Detection and characterization of aluminium-containing nanoparticles in a complex food matrix

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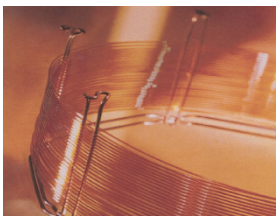
November 3–6, 2015
Prague, Czech Republic

Jana Pulkrabová, Monika Tomaniová, Michel Nielen and Jana Hajšlová
Editors



UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

RIKILT WAGENINGEN UR

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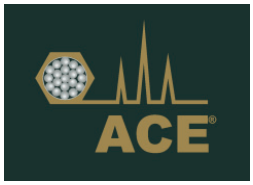

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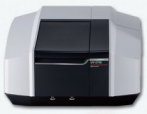

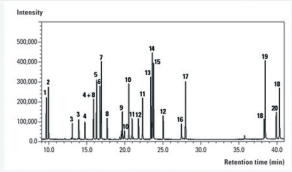

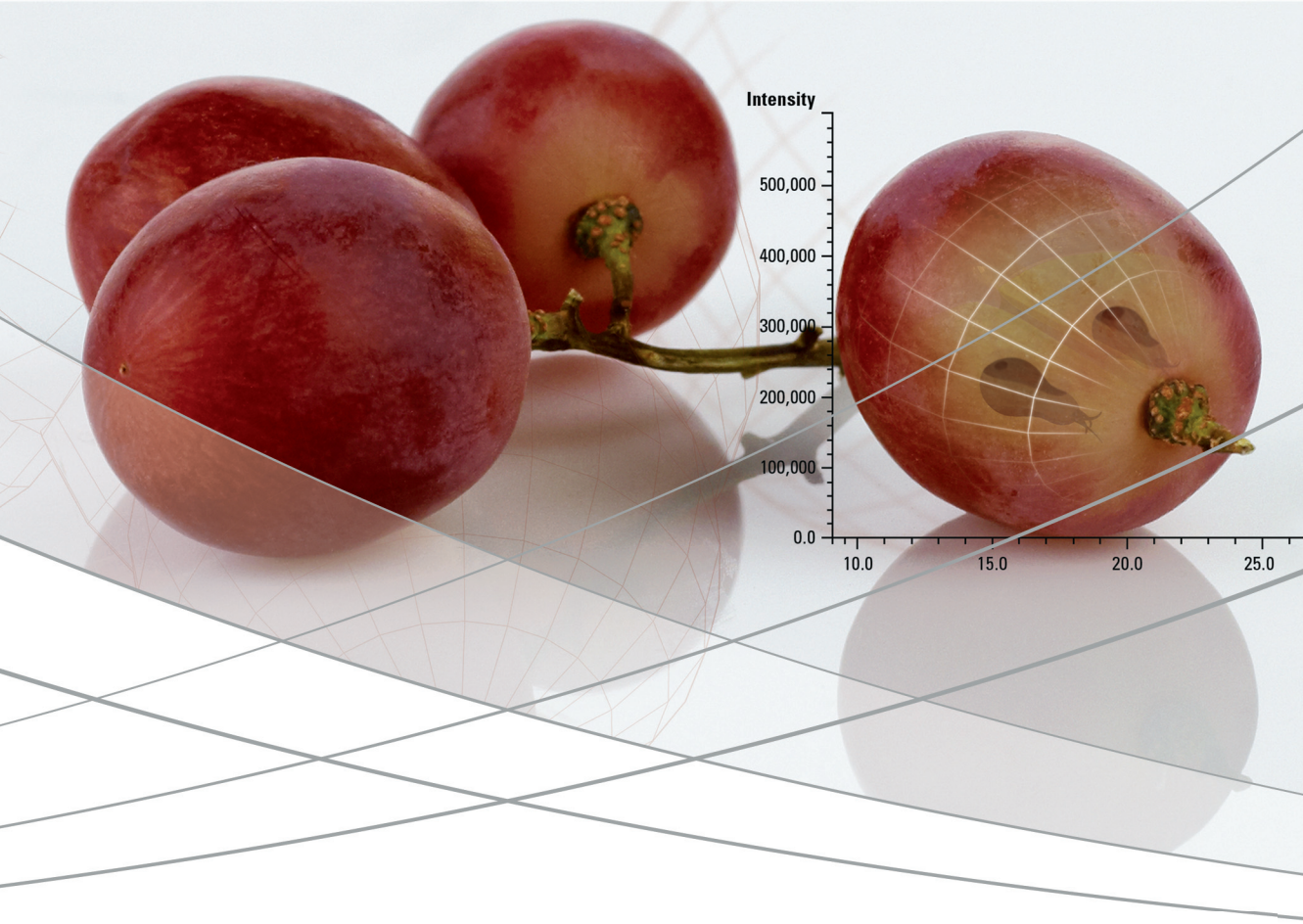

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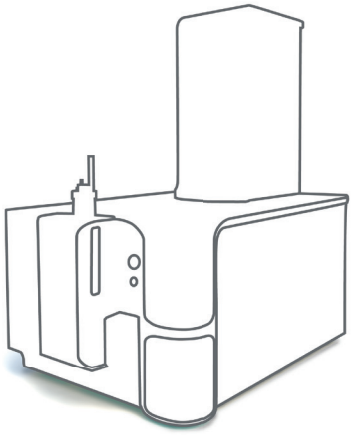

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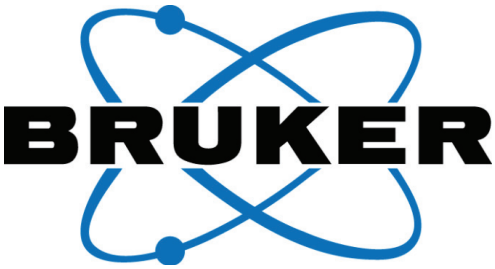
VENDOR SEMINARS

November 3, 2015 (12:30–13:30)

Elemental and Mass Spectrometric Solutions for the Analysis of Toxicants



Introduction to Bruker Daltonics solutions for food safety testing

Tony Drury, *Bruker Daltonics, UK*

Analysis of α-amanitin, β-amanitin and muscarine mushroom toxins in urine by UHPLC-QTOF mass spectrometry

Jana Tomkova, *Department of Forensic Medicine and Medical Law, Faculty Hospital Olomouc, Czech Republic*

For centuries, mushrooms have been a popular and often indispensible ingredient in the most exquisite cuisines around world. Their taste is unique, their caloric value is low and they are full of vitamins and minerals. Here, in the Czech Republic mushrooms grow everywhere and consequently mushroom picking is very popular and widespread, so much so that there is a common belief that mushrooming is a Czech 'national sport.' However, some varieties of mushroom contain lethal toxins and the most common cause of mushroom poisoning occurs through inadvertent misidentification of the varieties picked. Mushroom poisoning through dietary error resulting in hospitalization after ingestion is occasionally confirmed, and in 2014 out of 28 cases two were found to be positive.

Muscarine is the principal toxin in fungi of the genus Inocybe, Clitocybe and together with isoxazole derivatives ibotenic acid and muscimol is also present in the genus Amanita (*Amanita pantherina, Amanita muscaria* and others). *Amanita pantherina* is often confused with *Amanita spissa or Amanita rubescens* and *Amanita muscaria* is abused for its hallucinogenic effects.

The *Amanita phalloides* poisoning is rare, but may cause severe or even fatal intoxication. Amanita mushrooms contain amatoxins such as α-amanitin and β-amanitin and phallotoxins such as phallacidin. *Amanita phalloides* is often confused with agaricus or russula aeruginea.

Muscarine was used as a diagnostic marker for poisonings of *Amanita pantherina* and *Amanita muscaria*. α-amanitin and β-amanitin were used as diagnostic markers for *Amanita phalloides* poisoning. Phallacidin was chosen as an internal standard (IS) because it is not absorbed from the intestine.

Aims:

The aim of the present study was to develop a fast and sensitive method for simultaneous analysis of α-amanitin, β-amanitin and muscarine in human urine by solid-phase extraction (SPE) and ultra-high-performance liquid chromatography coupled with ultra-high-resolution TOF mass spectrometry.

Screening dioxin and pesticide residues in food extracts using GC–APCI coupled to high-resolution QTOF Mass Spectrometry

***Carsten Baessmann**, Bruker Daltonics, Germany*

The use of accurate mass QTOF–LC/MS with electrospray ionization for target pesticide screening enables the identification of hundreds of pesticides in a single run. On the other hand, GC/MS is well suited to these compounds and generally exhibits less matrix effects whilst producing lower chemical background. GC/MS is also well suited for trace analysis of other compound classes like polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF) and polybrominated diphenyl ethers (PBDE). As they belong to the class of persistent organic pollutants (POPs) they are one of the major concerns in present environmental discussion. Due to the accumulation in the food chain it is of general interest to analyze them with good sensitivity and confidence.

For pesticide analysis a standard mix consisting of 60 representative pesticides was spiked into fruit and vegetable matrices selected according to their relevance in food analysis and and their varied physiochemical characteristics, such as molecular mass, chemical composition, polarity and volatility. The mix contained amongst others: azinphos-Methyl, chlorpropham, diazinon, dimethoate, EPN, imazalil, myclobutanil and pirimicarb. 1 µl of each sample was injected and separated using a Restek Rxi-5ms capillary (30 m, 0.25 mm ID, 0.25 µm film). The GC column was interfaced to a Q–TOF–MS (Impact II, Bruker Daltonik GmbH) with a GC–APCI source operated in both positive and negative ionization modes. Data were acquired from 50–1,000 m/z at minimum of 4 Hz. All files were acquired with automatic mass calibration at the beginning of each GC/MS run with a perfluorinated calibration standard. All data were processed with the TASQ 1.0 software (Bruker Daltonik GmbH). The GC–APCI–Q–TOF–MS system was calibrated for quantification with the 60 pesticide standard in the concentration range of 0.05 to 500 pg/µl. Limits of quantification (LOQ) for most of the pesticides were found to be in the range well below 10 pg/µl with RSDs between 5 and 10% (N=3).

As key substances for POPs we analyzed decabromodiphenyl ether (DecaBDE) and 2,3,7,8-tetrachlordibenzodioxin (2,3,7,8-TeCDD) with the GC–APCI–Q–TOF–MS setup. PBDEs are among the EU priority substances. DecaBDE is the most difficult PBDE to analyze, because it is less volatile and additionally thermolabile. DecaBDE showed a good response at a concentration of 1 pg on column, LOD was even lower. The analytical working range was between 1–40 pg on column.

2,3,7,8-TeCDD is the most toxic substance of the PCDD/PCDF compounds. 2,3,7,8-TeCDD was detected as [M]+ signal and the LOD of 2,3,7,8-TeCDD was <0.1 pg on column. The calibration curve showed an analytical working range between 0.1–2000 pg on column. Using GC–APCI coupled to high resolution QTOF–MS we achieved excellent detection limits at the relevant environmental maximum residue limit levels.

A rapid and cost-efficient method for the measurement of Arsenic in rice

***Armin Gross**, Bruker Nano, Germany*

Almost half of the world's population eats rice every day, in many cases several times per day. Therefore, it is considered that rice is the dominant source of inorganic arsenic in the human diet. Rice contains significant amounts of inorganic arsenic with concentrations often between 0.1 to 0.4 mg arsenic/kg dry mass or higher.

Although it is known that chronic arsenic exposure is linked with cancers of the bladder, lungs, skin and prostate, as well as heart disease, only a few countries have established maximum levels (MLs) for total arsenic in food. In 2011 the World Health Organisation (WHO) has published a recommendation showing a maximum level of 200 µg/kg of inorganic As in polished rice.

The accurate detection if low levels of As in food by common atomic spectroscopy methods requires a dedicated laboratory infrastructure with sample preparation equipment (microwave digestion), cooling water and gas supplies. In contrast to that Total Reflection X-Ray Fluorescence (TXRF) spectrometry is an easy-to-use method which requires minimum sample preparation and no gases or any other media for an accurate multi-elements analysis in the ppb-range.

This paper describes the successful application and method development for the measurement of As in rice by TXRF. The new S2 PICOFOX Ultra Efficiency with a 50W micro focus tube and a 60 mm² XFlash silicon drift detector offers detection limits below 50 µg/kg after a rapid sample preparation without any digestion.

November 3, 2015 (12:30–13:30)

VENDOR SEMINAR:

A Challenging Story of GC-TOF MS: Technology Milestones and Two Amazing Decades of its Application in Food Analysis

Although the basics of Time of flight mass spectrometers (TOF MS) were established in 30s of 20th century, due to the lack of fast electronic the renewal of this technology was postponed to early 90s of last century. At this time the enormously fast, but extremely sensitive, first commercially available GC-TOF MS were introduced to the market. The nature of TOF MS technology, such as fast and sensitive acquisition of unskewed MS spectra, was followed by implementation advanced mathematical algorithms of data mining – automated peak find, deconvolution and scripting.

The potential of marketed fast GC-TOF MS instruments equipped with ion source not requiring its cleaning was later on extended by their combination with comprehensive gas chromatography (GCxGC).

The increased nowadays demands for analytical instrumentation can be summarized as: “One run covering all analyst’s requirements, such as target and non-target screening, qualitative and quantitative capabilities along with easy and fast hardware-software handling and reasonable data file sizes”. Such requirements were kept in mind within the GC-HRT instrument development employing the multi-reflection TOF MS analyzer (so called Flight Folded Path, FFP™). The GC-HRT routinely achieving mass resolution more than 50.000 FWHM and mass accuracy lower than 1ppm was recently upgraded with the GCxGC option extending its capabilities to the edge of possible – significantly enhanced separation combined with ultra-high resolution and ultimate mass accuracy.

Register and join the fantastic selection of TOF MS applications in food analysis presented by great scientists and excellent speakers in the same time.

Black pepper authenticity testing based on SPME-GC-TOF MS

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Black pepper is valuable commodity on worldwide market and may become a subject of fraud. Cases of adulteration of ground black pepper with cheaper plant materials or mislabeling have been encountered. Smart approaches for the testing of black pepper authenticity are needed to disclose such practices and protect consumers. One of conceivable authentication strategies is profiling of black pepper volatiles.

The evaluated set of samples in our study consisted of 16 ground black peppers and in addition to these samples, other materials derived from pepper (oleoresin, spent – residual material of oleoresin production, pepper peels), that can be under certain conditions used for adulteration, were delivered by spice trading company. Beside of these samples, twelve more pepper samples were collected in retail markets in the Czech Republic.

In this study, black pepper volatile profiles were obtained using head-space solid-phase microextraction coupled to gas chromatography - mass spectrometry. Time of flight mass analyzer equipped with automated deconvolution & peak find algorithm was used for primary data acquisition (TruTOF, LECO, USA). Using the

Statistical Compare feature of the ChromaTof software by LECO, compounds were aligned in all off measured samples and after the normalization of their areas, the statistical analysis was performed in Simca software (Ulmetrics).

All the samples, both labeled as authentic pepper by our commercial partner and those from retail market grouped together using principal component analysis. Other 3 samples separated clearly. For one of "suspect" samples, organic solvents used for oleoresin isolation were the most decisive compounds. Other separated samples showed similar profiles of volatiles to those of pepper related materials mentioned above.

Two-dimensional gas chromatography with time-of-flight mass spectrometric detection for determination of prohibited substances in food supplements

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In recent years a number of food supplements promising positive health effects, improving body shape and/or enhancing strong and endurance etc. have been introduced to the worldwide market. Food supplements include wide range of products containing various ingredients like herbs, herb extracts, vitamins, minerals, amino acids, proteins etc., however some of them can be contaminated by prohibited substances. Based on our 10 years experiences a number of compounds like anabolic steroids, tetrahydrocannabinol (THC) or even prescription drugs coming from cross-contamination or intentional manufacturer use can be found in these products.

To detect and identify unknown compounds in samples, non-target screening procedures based on full mass spectra measurement represent a feasible solution. For compounds that can be analysed by GC, two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOF) shows a method of choice. Enhanced sensitivity and chromatographic resolution resulting from separation of sample in two capillary columns with different polarities as well as unique identification of compounds due to the collection of full mass spectra are well known characteristics of this sophisticated technique.

Theoretical aspects together with practical examples and results of non-target screening of samples collected from Czech market during official control demonstrating potential of GCxGC-TOF will be presented.

Unknown off-flavor in plastic products of daily use – can HR TOF MS help to discover it?

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Odor emissions from plastic materials employed for production of various articles of daily use may represent a serious problem resulting in customers'´ complaints and, consequently, producers´ losses of market position. The identification of such ´smelly´ compounds and explanation of their release is a real laboratory challenge; since in many cases, they are present at (ultra)trace level, nevertheless have a very low odor threshold.

Non target screening of headspace volatiles employing solid phase microextraction coupled to gas chromatography / mass spectrometry (SPME-GC/MS) is a technique of choice. For this purpose, instrument equipped with high resolution time of flight mass analyzer (HR TOF) is the most suitable, since it enables acquisition of full high resolution spectral information, deconvolution of components occurring in complex mixtures. Identification / confirmation is then based spectral similarity, mass accuracy of detected ions and their isotopic pattern.

In a particular, two case studies will be presented:

i) Identification of compound causing musty odor in plastic inhaler

ii) Identification of compound causing "chemical"´ smell of plastic kettle.

Application of GCxGC-HR TOF MS in food and food-related matrices

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Nowaday demands in the food analysis (but also other matrices) require highest level of confidence at trace and even ultra-trace levels. High resolution instruments are successfully delivering such information and easily enabling the confirmation of analyte's identity. The difficulties can be observed, when non-target analysis is required in complex matrices, such as tea extracts.

The experience of analysts across the globe has proven that the two-dimensional comprehensive GC separation (GCxGC) along with reliably fast MS detection can solve the "complex matrix" issues. A hyphenation of GC and HR TOF MS is then the logical combination for delivering the right results.

Within this contribution the examples of advantages of GCxGC-HR TOF MS on real-life sample examinations will be demonstrated.

„Let the Robot do it"
Automated Sample Preparation for Food Analysis-related matrices

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When it comes to determining chemical compounds in food or beverages, a laboratory must have dependable analytical tools and capabilities. Food toxins, environmental pollutants in food, flavor interaction, compound adulteration or odor threshold,- what do all these things have in common? - The need for analytical precision, dynamic range and sensitivity. GERSTEL is a recognized expert in these areas.

Our expertise in automated sample preparation and in LC/MS and GC/MS analysis makes GERSTEL your source of solutions in the food and beverage industry as well as in the field of health and food safety.

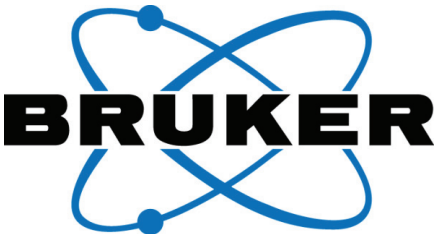
From an application focused on individual tasks such as liquid/liquid extraction to vortexing or sample weighing to a more complete sample preparation protocol, for example automation of QuEChERS, GERSTEL welcomes you to discuss the possibility for automation.

The complete GERSTEL product portfolio is specifically designed to meet your demand for high throughput and to enable modularity which might be necessary for new challenges in your analytical laboratory. GERSTEL offers a range of tools and techniques that are designed to meet customized challenges in the field of health and food safety laboratories.

Let us know how you do it.

November 4, 2015 (7:30–8:30)

Mass Spectrometric Solutions for the Analysis of Dioxins, Veterinary Drugs and Pesticides in Food and Feed



Analysis of dioxins according to Commission Regulations (EU) 589/2014 and (EU) 709/2014 by GC–Triple Quadrupole Mass Spectrometry

Gordon van't Slot, *Bruker Daltonics, Germany*

Already since 2012 it has been allowed to perform screening and quantitative measurements for dioxin analysis on low resolution mass spectrometers. In 2014 two new European Regulations for Food and Feed have been set into action allowing also confirmation of positive findings by TQ Mass Spectromety. 12 instead of high resolution mass spectrometry if you can show the compliance with some analytical criteria since 2012 already.

The following criteria must be fulfilled by the method:

- Each group requires at least one ^{13}C-labelled homologue per group of tetra- to octachlorinated PCDD/PCDF.
- Recovery of internal standards has to be between 30% and 140% for screening methods.
- Separation of the isomers 1,2,3,4,7,8 and 1,2,3,6,7,8-HxCDF has to be sufficient (<25% overlay peak to peak).
- The calibration curve has to cover the relevant concentrations starting from the level of detection.

Additional requirements which have been laid down with Regulations EU 589 and 709/2014 method requirements for confirmation of dioxins, and related compounds are

- Unit resolution for both analytical Quadrupoles
- Ion ratio tolerance <15%
- At least two significant precursor with one significant product ion each

Based on data from reference material in different matrices we are going to show, how modern high precision Triple Quadrupole mass spectrometers perform in these tasks. The compliance with all requirements is going to be shown on samples from food, feed and environmental with certified reference material provided by the Institute for Reference Materials and Measurements (IRMM). Pitfalls and possibilities are covered in this overview.

Screening and identification of veterinary drug and pesticide residues in food extracts by LC–QTOF Mass Spectrometry

Carsten Baessmann, *Bruker Daltonics, Germany*

Rapid, comprehensive screening for residues using full scan accurate mass has become a powerful tool in facilitating food safety monitoring. In addition to the high number of possible target compounds, the technique enables unknown screening and retrospective analysis. We describe the development of a solution for screening and quantitation of pesticide and veterinary drug residues in food matrices using a high-resolution LC–QTOF accurate mass system. Central parts of the solution is a newly developed software package (TASQ™: Target Analysis Screening and Quantitation) coupled with a high quality accurate mass and retention time database. TASQ software allows the simultaneous quantitation and confirmation of hundreds of pesticide residues by processing qualifier ions and subsequently applying a 'diagnostic ion'

confirmation criterion to their detection. The pesticide database contains over 700 pesticides including, retention time, exact mass of precursor ions (MS mode) and broadband CID ions (MS/MS mode) that have been annotated with molecular formulae. We show the simultaneous quantitation and identification of approximately 500 pesticides in food extracts. The pesticides were matrix matched in a dilution series (0.1–2000 µg/kg) using QuEChERS extracts: tomato, summer squash, potato and orange). The matrix matched calibrants were analyzed using a Bruker LC–QTOF system under a 15 minute reverse phase UHPLC gradient. Data acquisition was performed in alternating full scan and bbCID fragmentation modes. Automatic data evaluation was performed using TASQ processing software. For confident identification we use retention time, precursor accurate mass, isotopic pattern and up to 3 qualifier ions in full scan and 7 qualifier ions in bbCID acquisition. As result of this the LODs and LOQs of the 500 pesticides in the different matrices were determined. This approach can be also applied to other residues in food matrices, by changing the compounds contained in the database. As an example we present in a second study focused on the qualitative multi-residue screening for approximately 140 veterinary drugs in milk and fish.

November 4, 2015 (13:30–14:30)

Three New Products for Reliable Pesticide Analysis: from Enhanced Sample Preparation to Sensitive Detection

Agilent Technologies

Three new products for reliable pesticide analysis: from enhanced sample preparation to sensitive detection

Derick Lucas, ***Christoph Mueller***, *Agilent Technologies*

The key to success in pesticide analysis is maximising the efficiency of all the steps involved in it. Agilent Technologies is proud to present in this seminar three new products that dramatically improve analytical efficiency.

A novel sample preparation for fatty matrices, together with a new rock-solid LC/QQQ and the latest advancements in GC/MS technology for a broad coverage of pesticides.

The three new products, together with innovative software workflows make pesticide analysis an easier, more robust task.

November 4, 2015 (13:30–14:30)

Approaching Routine Exhaustive Organic Contaminant Screening with Innovative LC/MS, GC/MS and Ion Mobility Technologies

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

A fast, quantitative and qualitative QECHERS based commodity independent multi residue analysis on a APGC–XEVO TQ-S (micro)

***Wim Broer*, Nofalab**, *Schiedam, Netherlands*

E-mail: wim.broer@nofalab.nl, Phone: +31620068355

A fast Quantitative and Confirmative Multi Residue Pesticide on a GCMS in different commodities in one generic method is often a challenge. At Nofalab we developed a commodity independent method for over 300 pesticides on a XEVO TQ-S in combination with a APGC. At Nofalab we employ the XEVO TQ-S in combination with a APGC (Atmospheric Pressure Gas Chromatography) already for over four years successfully. We developed a standard extraction method for all food and feed commodities based on QUECHERS. Using the benefits of the soft ionisation of an Atmospheric Pressure Chemical Ionisation technic (APCI) such as the APGC where M^+ and MH^+ ions are abundant with minor fragmentation. A high sensitivity is reached by using this ionisation technic. Defining MRM's on EI-ionisation is often based on fragments which are not specific for one compound. In contrast to EI-technics with APCI MRM's can be based upon the M+ and/or MH+ ions giving a better specificity. Based upon this excellent performance gave the opportunity to modify the QUECHERS-extraction in such a way that a stable chromatographic separation is obtained and matrix interference is minimized. This results in a commodity independent method for the determination of pesticides. Using a XEVO TQ-S this method was validated for 300 pesticides in a 30 minute injection to injection method. Recently Nofalab acquired a XEVO TQ-S Micro. Compared with the TQ-S this instrument has a significantly faster scanning rate. In ESI-mode the sensitivity of the instrument is significantly lower, but is APGC–mode the sensitivity is comparable since in contrast to the ESI all the effluent of the GC-column is extracted into the Mass Spectrometer. In this presentation the optimization of the APGC is discussed based on 25 pesticides reflecting several behaviours such as injection stability, resolution, M^+, MH^+ and fragment formation on a XEVO TQ-S and the new XEVO TQ-S micro. The presentation concludes with the results of a validation of a quantitative and qualitative method for over 500 pesticides (all with 2 or more MRM's) on a XEVO TQ-S Micro within à 30 minute injection to injection run. For most pesticides the LOD is less than one fifth of the required MRL's are reached, for some even lower than 0.001 mg/kg with acceptable recoveries of 70-120% and a RSD below 20% as required by SANCO

Using a dynamic trio to empower your screening assays

***Séverine Goscinny*[1]**; *Michael McCullagh[2]*

[1]*Scientific Institute of Public Health, Brussels, Belgium*

[2]*Waters, Manchester, UK*

A novel new screening strategy for monitoring pesticides in foodstuffs has been developed. Increasingly, laboratories are performing preliminary low-cost, high sample throughput screening methods, using high-resolution liquid chromatography hyphenated to a full scan mass analyser (e.g. Time-of-Flight). Typically the list of targeted compounds is significant. After detection, the identification process starts and relies on different criteria with associated tolerances, to ensure low false detection rates. It is known, many factors can influence mass spectra for LC-based methods. With analysis of complex samples, reliable identification can be unreachable. Ion mobility can reduce sample complexity, whilst increasing specificity. We will present a

novel way to use ion mobility features routinely and illustrate how robust CCS values can efficiently reduce false detection rates.

UPLC ion mobility data covering a mass range from m/z 50 to 1200, was initially acquired for a series of solvent standard mixtures. These were utilized to generate mobility separated single component precursor/fragmentation spectra for the M^+H^+ or adducted species. An in-house CCS library was built and inserted into the scientific library, used for automatic software screening. Initial feasibility studies ware performed on five different instruments to assess the robustness of the CCS values generated. Further experiments were repeated on different days, during 10 months. Matrix and concentration studies were performed using blank and fortified matrices, processed with a methanolic extraction method, without any purification step. The overall CCS screening performance was evaluated using previous proficiency tests.

CCS values for 150 pesticides were measured by UPLC–HDMSE experiments using solutions in solvents at 100 ng/mL.These values were inserted in an in-house library with associated retention times, accurate mass and diagnostic fragments. Impact of analyte concentration on CCS was assessed by analyzing standards solutions from 1 ng/mL to 100 ng/mL. These results show that at any concentration there is no more than 2% difference with the values in the library. The same protocol was applied to samples with increasing matrix content (10 g, 40 g and 60 g of sample take) and the CCS generated; demonstrate no more than 2% error. These observation clearly indicate that targeted compound and sample concentration do not impact CCS, contrary to retention times and mass accuracy in LC-MS based methods. Furthermore, the reproducibility of CCS values were studied by comparing values obtained on different days, before and after a large batch of analyses, also data acquired for a period time of ten months. The RSDs calculated for these CCS are lower than 1%. In addition, CCS values generated from five different analytical platforms were of the order of 2% compared the library values. These findings raised the possibility that CCS can be used to help the identification process of targeted compounds, and were inserted in a routine workflow for pesticide screening. To test the method, a previous proficiency test was analysed and we will demonstrate how CCS used as an identification parameter, decrease false positive and most importantly avoids false negatives, even with less stringent screening parameters. We will also discuss, detection based on monoisotopic peak information, where CCS values within 2% tolerance confirm identification and avoid false negative reporting. The robustness of CCS is further illustrated when comparing a new travelling wave IMS-QToF ion mobility platform.

Novel Aspect:

CCS used as a new point of identification in routine full scan screening assays using travelling wave ion mobility platforms.

November 4, 2015 (13:30–14:30)

From Sample Extraction to Data Analysis of Complex Samples with Smart Solutions

Odors from food packaging – an analytical challenge?

Erich Leitner, *Technical University Graz, Graz, Austria*

The smell of a product (food or non-food) is one of the most influencing parameters for the acceptance or the rejection of products by consumers. In addition the quality of food packaging material have to meet the general requirements stated in the Framework Regulation (EC) No. 1935/2004 where it is clearly defined that "*…they do not transfer their constituents into foodstuffs in quantities which could endanger human health or bring about an unacceptable change in the composition of the foodstuffs or deterioration in the organoleptic characteristics thereof*". The olfactory sensation is processed in the limbic system, which is highly linked to emotions and therefore highly subjective. A more objective approach is the analytical determination of odor active substances by gas chromatographic techniques; because only volatile substances in a molecular weight range of up to 300-400 Dalton can show odor activity. Nevertheless, for setting up analytical methods for odor active substances the methods must be able to reach the concentration ranges of the sensory thresholds. Several examples for the identification and quantification of different packaging materials will demonstrate the use of one and two dimensional applications of GC-MS and GC-MS/MS.

Innovative strategies for Dioxins/PCBs extraction and purification

Philippe Marchand, *LABERCA, Nantes, France*

LABERCA, French Reference Laboratory for Dioxins and PCBs, in collaboration with BUCHI, analyzed different samples from international PTs in dioxins and PCBs. Samples were extracted using the SpeedExtractor, purified on a new automatic system (MIURA GX-300) and analyzed by GC–HRMS (Jeol). This new technique increases productivity by processing up to 6 samples in parallel and provides satisfactory results in terms of repeatability, reproducibility and accuracy.

Lipidomic analysis by using one- and multidimensional chromatography coupled to mass spectrometry

Francesco Cacciola / ***Luigi Mondello***, *University of Messina, Messina, Italy*

Lipidomics is a **branch** of metabolomics and aims to study all the lipids within a living system or in complex biological samples. The abundance and differences of individual lipid molecular species may be indicative as a clinical tool for risk assessment and disease monitoring. In this contribution, a practical workflow for lipid profiling of biological and food samples by one- and multidimensional chromatography coupled to mass spectrometry (MS) is illustrated. In particular, the analysis of intact lipid constituents was carried out by reversed-phase (RP) liquid chromatography and silver ion chromatography (for a class-type separation) coupled to RP-LC (for lipid species separation).

November 4, 2015 (13:30–14:30)

Elastic Light Scatter – A New Technology for Rapid Identification of Pathogens

Introduction and background to the elastic light scatter (ELS) technology

J. Paul Robinson, *Purdue University, West Lafayette, USA*

Techniques for rapid identification of microorganisms cover a variety of technologies. Depending upon the use environment, certain features may be more important than others to the user. The technology we will describe has a number of advantages since it is categorized as reagent-free and is based on identification of organisms based on colony feature space. The technology is based on the use of elastic light scatter (ELS) whereby a laser beam strikes a colony on an agar plate and the resulting scatter pattern produced becomes a distinctive fingerprint for that organism. This fingerprint is created by establishing a classifier based on having a series of identified organisms and creating a number of known organisms. Once the system has been trained, the user can identify those organisms rapidly and in a reagent free manner. This workshop will outline the fundamentals of ELS technology, we will provide some insight into the core of the optics and physics of how ELS works and we will also discus how the classification technology operates. Finally we will outline applications and results of a number of tests that the technology has undergone.

The physics and optics of ELS

Euiwon Bae, *Purdue University, West Lafayette, USA*

The remarkable resolving power of the elastic light scatter (ELS) originates from the phenomena called the optical interference. ELS works by encoding the biological characteristics (micro- and macro-structural morphology) into optical signals of an interrogating wave front. With high coherency and nanometer wavelength, the incoming plane wave passes through the bacterial colony from top to bottom. Various physical parameters–such as refractive indices, the local density of bacteria and the individual shape of bacteria–can all influence the incoming photons. This interaction through the z-depth of the colony accumulates and finally disrupts the amplitude and phase of the incoming photons. The propagating lights will either result in constructive (bright spot) destructive interference (dark spot) based on the spatial distribution of the morphological and material characteristics. These secrets are then decoded through examination of the forward-scattering pattern. The use of a spatial light modulator (SLM) and a liquid-crystal display represents a renowned famous technique used in optical engineering to control wave front modulation. We can understand the photon-colony interaction as a “biological SLM” that changes the wave front characteristics based on the colony’s physical differences.

The principles of recognition and classification of ELS patterns

Bartek Rajwa, *Purdue University, West Lafayette, USA*

The major difficulty posed by ELS measurements lies in deciphering the highly complicated ELS patterns formed by bacterial colonies irradiated with laser light. Even though the well-developed light-scattering theory and accompanying computational tools such as dipole-dipole approximation could be used for modeling and subsequent interpretation of the raw ELS signals, such a rigorous approach to the inverse-scattering problem remains extremely difficult and computationally expensive. This presentation will discuss a robust and rapid alternative methodology for pathogen recognition taking advantage of machine-learning (ML) and computer-vision tools for classification of ELS patterns formed by interaction between laser light and colony morphotypes. The employed classification algorithms (such as SVM, NN, etc) do not operate on raw ELS patterns, but utilize complex moments that are calculated in the polar coordinate space of the patterns as

input features. The results demonstrate the use of the ML–ELS to classify colonies of *Listeria*, *E.coli,* and *Salmonella* with accuracy above 95

Summary of Applications

***J. Paul Robinson**, Purdue University, West Lafayette, USA*

Elastic light scatter has been proven to be a successful detection and identification technology for pathogen identification focusing heavily on food borne pathogens. Over the past several years during the development of the technology, the development groups have published over a dozen peer reviewed papers as diverse as the fundamental optics, computer science, and of course the microbiology. The first paper published on this technology focused on fundamental math describing the technologies needed to create a classification[(1).] Subsequent papers demonstrated an entire operating system of sample collection, analysis, classification and identification using Listera, Salmonella, Staph and Enterobacter as examples[(2).] The technology rapidly advanced to studying food contamination directly to show to ability to extract and identify food borne pathogens [(3)] and was extended to a variety of Vibrios [(4),] Salmonella [(5)] and even to portable instrument design[(6).] This presentation will walk through the past 9 years of research and development that have driven the development of this innovative technology

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[2] Biosensors and Bioelectronics 22 (2007) 1664–1671.
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November 4, 2015 (18:30–19:30)

Mycotoxin Analysis in Your Hand

Mycotoxin analysis in your hand

Christine Gutschelhofer*, *Ronald Niemeijer, *R-Biopharm AG, Darmstadt, Germany*

Mycotoxin contaminations of food and feed have a huge economic impact. Mycotoxins impose a risk to human and animal health. Therefore maximum limits have been established for many commodities. Legislations and guidelines are implemented and enforced in most parts of the world.

Since mycotoxins are natural occurring toxins, they cannot be avoided. As a result significant amounts of commodities are discarded or used for feed or non-food applications at a lower sales price. Financial losses however go far beyond the value of the contaminated commodities and may actually affect the entire food production chain. Animal feed contaminated with mycotoxins may cause production losses in livestock production and mycotoxins may cause significant health costs.

Mycotoxins contaminations of crops are unavoidable but mycotoxins can be managed. Good agricultural and good manufacturing practices will help. Monitoring mycotoxin contaminations by testing is necessary to verify the products will meet international regulations and guidelines. Yet, instead of testing large numbers of end-products, a more pro-active approach would have many benefits.

During the entire process from field to food or feed critical steps can be identified to monitor mycotoxins. For this approach a mobile, easy to use tool to make quick, on-site decisions is essential. In this workshop R-Biopharm will present the next generation in rapid, on-site mycotoxin testing.

November 4, 2015 (18:30–19:30)

Exploiting Alternative Selectivity to C18 Stationary Phases in HPLC

Exploiting alternative selectivity to C18 stationary phases in HPLC

Brian Kinsella, *UCT Inc., USA*

C18 remains the most widely used stationary phase in high-performance liquid chromatography (HPLC).

Numerous advancements and modifications have been made to both stationary phases and the underlying silica support particles over the years. Notable commercial introductions include hybrid particles, types of bonding (monomeric vs. polymeric), improved carbon loading and degree of endcapping. These modifications can offer improved retention of moderately polar analytes, decreased tailing of basic analytes, and permit use of higher pH mobile phases. The introduction of polar modified C18 stationary phases (or aqueous C18) containing polar side chains, embedded polar groups or polar endcapping has further improved the retention and peak shape of moderately polar compounds. However, challenges still remain in obtaining adequate retention and/or peak shape of more polar compounds, especially for small basic compounds that are used with low pH mobile phases that are typically used in LC–MS analysis.

Although alternative sorbent chemistry, and selectivity, are regularly exploited in solid-phase extraction (e.g. polystyrene-divinylbenzene) the same cannot be said for HPLC. However, HPLC columns that contain alternative functional groups are commercially available and can be readily used to address some of the difficulties that chemists often experience with standard alkyl phases. Examples of such functional groups include phenyl, polyaromatic and pentafluorophenylpropyl (PFPP) stationary phases. Theses HPLC columns exhibit alternative/orthogonal selectivity to C18 phases and can be used for compounds that are hard to resolve or that are unretained on C18 columns, particularly aromatic compounds. These phases can retain analytes through π- π and hydrophobic (dispersive) interactions, while the PFPP phase also exhibits dipole-dipole and H-bonding capability and can strongly retain basic, halogenated and nitrogen-containing compounds. Furthermore, due to its polar and non-polar character, the PFPP phase can exhibit dual-mode retention behavior (reversed phase and HILIC–type retention of basic compounds).

This presentation will discuss the use of aromatic HPLC columns and how they can be an optimal choice for use with conventional LC–MS solvents and mobile phase additives for the analysis of a wide range of compounds, including pesticides, veterinary drugs, mycotoxins and environmental contaminants.

November 4, 2015 (18:30–19:30)

Advancements in Pesticides Analysis (LC/GC/Sample Prep)

Advancements in pesticides analysis (LC/GC/Sample Prep)

Andrea Gheduzzi, *Phenomenex, Italy*

Historically, pesticide testing has been driven by regulatory requirements in sample preparation, GC and LC techniques.Traditional regulatory approaches in the testing of pesticides, herbicides, PCBs, and related compounds have been sufficient to achieve minimum method goals. However, recent technological and intellectual advancements in chromatography now provide more choices and options to improve your analytical success. This presentation provides an overview and a perspective on different options that are often overlooked for pesticides testing.

November 4, 2015 (18:30–19:30)

Learn More About Food Safety Solutions & Innovations from SCIEX

Tips to reduce matrix effects, increase throughput, and decrease data processing time for routine food testing

Andre Schreiber, *SCIEX, Concord, ON, Canada*

Mass spec is very common in routine food testing, but labs are still facing challenges to meet the ever-growing demands of food safety testing. Complex foods continue to produce matrix effects that can create highly unreliable quantitative results. Many chemical compounds must be analyzed by single-residue methods, adding significant time for analysis when multiple methods must be run on each sample. And, with multi-residue methods easily logging 100s of compounds, data processing across large batches of samples can take hours or even days. It's time to address these challenges head-on. This seminar will present new LC–MS/MS tools, tips, and workflows designed to help food testing labs overcome these and other every-day obstacles. New innovations in MS developments will also be highlighted.

November 5, 2015 (13:30–14:30)

Simultaneous On-Line Detection of Si, Ti and Al-Containing Particles in Toothpaste by Asymmetric Flow Field-Flow Fractionation Coupled with ICP–QQQ–MS

Agilent Technologies

Simultaneous On-Line Detection of Si, Ti and Al-Containing Particles in Toothpaste by Asymmetric Flow Field-Flow Fractionation Coupled with ICP–QQQ–MS

Katrin Loeschner, *Manuel Correia, Erik H. Larsen*

National Food Institute, Technical University of Denmark, Denmark

Toothpaste is a complex mixture of chemicals and includes surfactants, whiteners and abrasives based on nano or micrometer sized SiO_2, TiO_2 and Al_2O_3. A fraction of toothpaste may be swallowed during its normal use and individuals may therefore be exposed to these metal oxides. The size of the particles is a determining factor for their biological fate and the possible intestinal uptake of these particles. Therefore, in order to characterize these nano or microparticles, a method development project was initiated aiming at simultaneous size separation of all three types of particles by asymmetric flow field-flow fractionation (AF^4). Multi angle light scattering was used for on-line size determination of the eluting particles, and ICP–QQQ–MS was invaluable for selective, simultaneous detection of all three elements under a fixed set of instrumental conditions. In this lecture, results on the AF^4 and the ICP—QQQ–MS optimization work will be presented along with fractograms of real toothpaste samples using the coupled AF^4-ICP–QQQ–MS system.

November 5, 2015 (13:30–14:30)

High Resolution Accurate Mass: 'Food for Thought'

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High resolution accurate mass: 'Food for Thought'

Michal Godula[1], Hans G J Mol[2], Marc Tienstra[2], Paul Zomer[2], Jana Hajšlová[3], Jana Pulkrabová[3], Michal Stupák[3], Dominic Roberts[4], Paul Silcock[4]

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INTRODUCTION

The introduction of high resolution, accurate mass (HRAM) Orbitrap™ Technology coupled to Gas Chromatography (GC) brings a new level of performance and flexibility in GC–MS full scan acquisition and more, and perfectly complements recent developments in new MS/MS acquisition modes using LC–Orbitrap. Together these technologies have the potential to provide truly comprehensive workflow solutions, whether profiling and characterising samples; or performing simultaneously quantitative target residues and contaminant analysis whilst screening for unexpected compounds in complex samples. Furthermore, this is all in compliance with international quality control criteria and all in a single analysis.

With this potential in mind, the presentation will firstly provide a brief overview of the performance and the advantages of the new technical developments using Orbitrap technology. The first results from a proof-of–concept study into the performance of Q-Exactive GC™ for the analysis of GC amenable pesticide residues in different food and feed samples, with a particular focus on selectivity and detection capability, will then be presented. Similarly the results from the analysis of the LC–amenable pesticides using the Q-Exactive operated in full scan and simultaneous MS/MS, focusing on the advantages of variable Data Independent MS/MS analysis (vDIA) for improved detectability and identification will be described.

The presentation will conclude with a preliminary assessment of the performance of Q Exactive GC™ in the characterisation and profiling of extracts of whisky samples. The importance parameters such as high resolving power, excellent mass accuracy, wide linear dynamic range and automated deconvolution of HRAM spectra for the analysis of chemical components present at high and low concentrations will be discussed. The long term objective is to use such characterisation data to identify counterfeit products and for quality control to ensure the consistency of products.

This presentation will hopefully provide you with much 'Food for Thought' across a wide range of potential applications.

November 5, 2015 (13:30–14:30)

Prolonging GC–MS/MS Performance: Shoot and Dilute Injection versus Analyte Protectants

Prolonging GC–MS/MS performance: shoot and dilute injection versus analyte protectants

Julie Kowalski*, *Jack Cochran, *Restek Corporation*

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In gas chromatography–mass spectrometry (GC–MS), most problems occur on the front end, at the GC inlet, where compounds can degrade during hot splitless injection, active compounds can be irreversibly adsorbed to inlet liner surfaces, and nonvolatile material from dirty samples can compromise the transfer of less volatile compounds of interest from the inlet to the GC column. These issues are magnified due to the very slow inlet flow during splitless injection, which is typically less than 2 mL/min.

Two strategies to mitigate these issues will be demonstrated in this seminar. One approach is to use split injection, what we call, “Shoot and Dilute”. With newer, more sensitive GC–MS/MS systems LOD and LOQ requirements are often achievable using split injections at ratios of 10:1 or greater. Increased flow through the inlet during split injection minimizes residence time inside the inlet liner, which decreases compound degradation and adsorption, and maintains acceptable data quality longer. In addition, GC oven start temperature can be higher thus reducing overall run time as well as the time needed to re-equilibrate the GC oven prior to the next analysis. Another benefit of split injection is improved peak shape for early eluting pesticides when injecting acetonitrile-based QuEChERS extracts.

The second strategy to overcome GC inlet problems is to use “analyte protectants,” which are essentially volatile and chromatograph-able masking agents such as sugars, diols, etc., that are co-injected with each sample and standard to temporarily occupy active sites in the GC inlet liner and column. These analyte protectants have low *m/z* ions and the mass spectrometer can essentially overlook them in favor of target compounds.

Both strategies were tested with multi-class pesticides and compared against a typical splitless injection method without use of analyte protectants for QuEChERS samples. For Shoot and Dilute, viability of split injection based on detectability of a wide range of analytes was determined. Optimized split injection, inlet and initial GC oven temperatures were determined. Benefits of analyte protectants were evaluated by peak shapes and responses of both well-behaved and problem pesticides. The goal of both Shoot and Dilute and analyte protectants approaches is to improve initial and long-term chromatographic performance.

November 5, 2015 (13:30–14:30)

Developments in Routine Mycotoxins Analysis

Masked mycotoxins: is it a real threat?

Chiara Dall'Asta, *Università degli Studi di Parma, Italy*

The detection of masked mycotoxins is a challenge for analysts. A number of papers have been published in which instrumental and immunochemical methods were employed to reveal hidden mycotoxins in food and feedstuffs. The necessity of detecting both native and metabolized mycotoxins takes origin from the possible release of the parent compound in the gastrointestinal tract of humans and animals. What about the prevalence of masked mycotoxins in real matrices? This presentation provides an overview of known occurrence data.

Routine analysis of DON, 3AcDON and DON3Glc by an immunoassay

Laura Righetti, *Università degli Studi di Parma, Italy*

Immunochemical test kits are commercially available in different formats for the detection of mycotoxins in different matrices. Although the recent EU 519/2014 requires only a qualitative performance verification, routine users need reliable and accurate results as well. Overestimations in respect of instrumental analysis are normally rejected by analysts, even if they could be linked to the capability of the assay to detect the native analyte and its metabolites. A proper management of screening data should be based on a complete knowledge of the performance of the test kit in matrix, despite the cross-reactivities claimed in the kit insert.

Multiresidual screening of mycotoxins, novel mycotoxins and masked mycotoxins what about the future of immunoassay?

Maurizio Paleologo Oriundi, *Tecna s.r.l., Italy*

Multiplex screening of contaminants has been subject of many recent publications. Discovering the whole profile of one single sample means giving a value to the sample itself, thus optimizing the costs of its collection and preparation. Coupled with proper extraction and clean-up, LC–MS is suitable for multiresidual characterization of contaminants. On the other hand, immunochemistry-based methods are possibly able to detect a family of structurally related compounds, although the characterization of single contributions is not possible so far. Due to the reasonable interference of the matrix and the uneven pattern of cross-reactivity, results could be hard to understand, since sometimes they correlate neither with one single contaminant concentration nor with sum of the whole group of molecules.

Still, immunochemical-base methods could conjugate, in the feature, the well known ease of use, fastness and cost-effectiveness of ELISA and lateral flows with the multiplexing nowadays possible with the expensive LC–MS techniques only.

LECTURES

(L1 – L131)

L1
FOOD ANALYSIS: INTEGRAL PART OF FOOD SAFETY POLICY IN A CHANGING WORLD (AN EUROPEAN PERSPECTIVE)

Ladislav Miko[1*]

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L2
ELLIOTT REVIEW INTO THE INTEGRITY AND ASSURANCE OF FOOD SUPPLY NETWORKS – FINAL REPORT; A NATIONAL FOOD CRIME PREVENTION FRAMEWORK

Chris Elliott[1*]

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In 2013 the horsemeat scandal first hit the UK then spread right across Europe. The beef supply chain of many European countries was found to be contaminated with horsemeat. Many consumers lost confidence in their national food supply systems. Professor Chris Elliott was asked by the UK government to conduct an independent review of the UK food supply system in order to determine what had gone wrong and to suggest measures to make the system much more robust from fraud. In September 2014 the Elliott Review was published. This document gave eight major recommendations to the UK government and food industry about how to deal with the serious issues of criminality in the UK food system. The report detailed a systems approach to food crime prevention. An overview of this systems approach and how it is currently being implemented will be given. Particular attention will be focused on how advances in analytical science can help combat food fraud.

Keywords: *food authenticity, food fraud, horsemeat scandal, food crime prevention*

L3
INSTANTANEOUS FOOD CHARACTERIZATION BY AMBIENT MASS SPECTROMETRY

Zoltan Takats[1*]

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Mass spectrometry has traditionally been one of the 'last resorts' for food analysis, both for establishing authenticity and detecting trace components. While isotope ratio MS, gas chromatography-MS (GC-MS) and liquid chromatography-MS (LC-MS) are widely used for food and agricultural product analysis, MS methods (including these) are generally considered to be slow, expensive and delicate for routine field applications, mostly due to laborious sample preparation procedures. The advent of ambient ionization mass spectrometric methods raised most of the constraints associated with sample preparation and opened new opportunities for in-situ analysis. Since ambient ionization MS (AIMS) methods do not require sample preparation, the use of internal standards (or even external calibrators) is often impossible, resulting in the lack of quantitative information provided by these methods. Nevertheless, the spectral profiles are highly characteristic for the type, origin, age, etc. of the sample, which makes these approaches excellent for profiling analysis. In these cases the MS spectral information is used as a 'fingerprint' for the identification of critical attributes. Rapid Evaporative Ionization MS (REIMS) was originally developed as a direct combination of electrosurgery (surgical diathermy) and MS, for the intraoperative identification of cancerous tissue and surgical margin control. However it has become clear already at the initial tests that the method can equally be used for the instantaneous characterisation of meat and fish as well as practically any water-containing food item. Applications have been developed for food adulteration testing (e.g. detection of horse meat in processed food) and establishing authenticity. Desorption Electrospray Ionization MS – the first AIMS method developed – gives a complementary alternative to REIMS. DESI is an excellent tool for the profiling of dry products (i.e. products not amenable to REIMS analysis) ranging from herbs, spices to seeds, chocolate, etc. DESI can also be used for the analysis of thin liquids (e.g. milk or wine) by combining it with Solid Phase Microextraction (SPME) or similar techniques. Direct analysis in real time (DART), Desorption Atmospheric pressure Chemical Ionization (DAPCI) and various plasma desorption techniques have also been used extensively for the detection of volatile components including pesticides and aroma compounds. All AIMS methods have already been combined with miniaturized/portable MS systems, which makes them excellent tools for the field testing of various food items from dairy products through meats and vegetables to alcoholic beverages.

Keywords: *ambient mass spectrometry, ionization mass spectrometric methods, miniaturized/portable MS systems*

L4
CHALLENGES OFFERED BY ION-MOBILITY MS TO FOOD CHEMISTS

Jana Hajšlová[1*], **Ondřej Lacina**[2], **Milena Zachariášová**[3], **Marie Fenclová**[4], **Vít Kosek** [5]

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Although ion mobility–mass spectrometry (IM–MS) is a technique known for decades, its applications in the field of food analysis have been introduced only recently. The principle behind gaseous phase IM separation of ionized analytes are differences in their migration through electric field. While most of currently available instruments provide space separation, some of them operate with a drift gas flow that provides a temporal separation. In any case, in the IM component, ions are separated according to their mass, charge, size and shape. Since IM separation typically occurs in millisecond timeframe and MS detection takes only microseconds, additional separation technique such as liquid chromatography (LC) can be hyphenated without compromising the speed of MS detection.
In this presentation, following powerful features of LC–IM–MS platform employing electrostatic drift tube IM and HR MS(/MS) mass analyzer will be demonstrated:

- Improved quality of mass spectral information obtained thanks to filtered background
- Simplified interpretation of mass spectra thanks to their ´logical´ nature (parents and fragments have common drift time)
- Separation of isomers of the same chemical compound which cannot be resolved by MS (and sometimes neither by U-HPLC)
- Enhanced analytes identification thanks to structural information (size and shape) based on collision cross-section measurement
- Improved quality of IM data (dynamic range, sensitivity) enabled by multiplexing

The case study made on complex matrices (whisky) will critically document not only potential but also limitations of LC–IM–MS when used for authentication purpose.

Keywords: *ion mobility Q-TOF LC/MS, whisky*

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L5
COMPLEMENTARY APPROACHES IN FOODOMICS TOWARDS NEW HORIZONS IN FOOD ANALYSIS

Michael Rychlik[1*]

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Since the advent of the concept "-omics" in "genomics", "proteomics" and "metabolomics" there has been inflationary use of this term. In case of foods, the "foodomics" were born and encompass all "-omics" methods in relation to the matrix food. A straightforward application of foodomics is elucidating or confirming the authenticity of foods. This term generally is related to one or more of the following attributes: geographic origin, type of agricultural production, species and kind of raw materials, or certain process qualities such as sustainability or ecologic foot print. In the recent years, there has been tremendous progress in high resolution methods to elucidate the molecular fingerprint of foods. On the genetic scale (genomics), apart from classical polymerase chain reaction, new developments of isothermal amplifications or next generation sequencing will enable more accurate identification of species. On the protein level (proteomics), specific biomarker peptides are being used. Further methods for profiling are assessing the ratios and positions of stable isotopes in marker molecules (Stable Isotope Ratio Analysis, isotopolomics) or ICP–MS of rare earth elements (metallomics). For a fingerprint of metabolites, the new methods of non-targeted and targeted metabolomics, e.g. via Fourier transform ion cyclotron mass spectrometry (FT/ICR–MS) or multidimensional NMR already allow a specific authentification of some food items. Apart from authenticity, non-targeted metabolomics may also open new avenues into safety evaluation of foods and food components. In this way, combination effects of contaminants may be monitored by their respective metabolic response or metabolomic approaches may serve to partly substitute animal testing. These and further developments in food analysis will be presented.

Keywords: *foodomics, food authenticity, profiling, fingerprinting, non-targeted and targeted metabolomics*

L6
COMPREHENSIVE CHROMATOGRAPHY (GC×GC, LC×LC) TECHNIQUES COUPLED TO MASS SPECTROMETRY FOR THE ANALYSIS OF FOOD SAMPLES

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Comprehensive 2D chromatography separations are performed on two different columns, with a complementary and as much as orthogonal separation capability. The transfer system (defined as modulator), is located between the two dimensions, and enables the continuous and sequential transfer of primary-column effluent bands onto the second column. Consequently, the entire initial sample is subjected to two separation steps. Comprehensive 2D chromatography technologies produce very high capacities since the resulting peak capacity is the product of the peak capacities relative to each dimension. The comprehensive 2D chromatography technologies, which will be the object of discussion, are based on liquid (LC×LC) and gas (GC×GC) mobile phases. With regard to mass spectrometry (MS) instrumentation, a great deal of evolution has occurred over the last 15 years; in particular, ultimate generation MS/MS systems can be employed for both untargeted and highly-selective/sensitive targeted analyses. These novel MS devices are capable to satisfy the requisites of both LC×LC and GC×GC separations, with such a combination creating highly powerful and flexible four-dimensional analytical tools. GC×GC–MS investigations were directed to experiments involving the analysis of phytosanitary compounds in drinking water. The number of possible combination of stationary phases is higher in LC×LC, with respect to GC×GC, in order to maximize the gain in peak capacity, through the coupling of independent separation modes. When a class-type separation is to be achieved in the first dimension, orthogonality may be obtained by using hydrophilic interaction liquid chromatography (HILIC), coupled to reversed-phase (RP) LC; this approach was applied for the characterization of the lipidic fraction of sea organisms. Whatever the front-end separation, the use of MS/MS brings in added dimensions in terms of selectivity, specificity, and structural information. Finally, a multidimensional LC–GC application with a double detection [flame ionization detection (FID) and triple quadrupole (QqQ) MS] will be illustrated for the determination of mineral oil contamination in edible oils.

Keywords: *comprehensive chromatography, contaminants, mass spectrometry*

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L7
WHAT IS THE ORIGIN OF THIS OLIVE OIL? SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) COUPLED TO QTOF–MS MAY PROVIDE A RAPID ANSWER

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A novel untargeted metabolomics approach using normal-phase (NP) Supercritical Fluid Chromatography / Quadrupole Time-of-flight Mass Spectrometry (NP-SFC/QTOF–MS) in combination with multivariate statistical analysis (MVA) will be presented.
The study was initially conducted to develop a dilute-and-shoot method authenticating olive oil samples with respect to their geographical origin. Olive oil has historically been one of the most frequently adulterated, counterfeited and sophisticated products. Apart from the financial crime aspect, such frauds can sometimes cause severe risks for the public safety not been observed the specific principles incorporated into an Hazard Analysis Critical Control Point (HACCP) system ensuring that the best possible hygiene and traceability practices have been followed along the processing line of virgin oil.
Olive oil lipid profiling on GC system often requires derivatization as part of sample preparation, while RPLC a phase transfer into a weaker eluent than mobile phase. Most importantly, the traditional RPLC and HILIC–LC methods use a mobile phase containing at least one organic solvent in high percentage and/or, to improve the retention, ion-pairing reagents such as trifluoroacetic acid, sodium octanesulfonate, hexanesulfonic acid.
In contrast, in NPSFC the solvent exchange is eliminated by injecting the organic phase diluted sample (or its extract) directly into a SFC system resulting in a most environment-friendly and cost-time approach due to the solvent reduction. Additionally, compared to HPLC–MS methods, the low viscosity of the SF, and consequently, the low height equivalent to theoretical plate (HETP) at higher flow rates, allow shorter analysis times and more selective detection of analytes in complex mixture (such as olive oil) when SFC is coupled with a MS detector.
We will present a metabolomics approach extended to 220 olive oil samples representative of the Mediterranean production. It allowed not only to discriminate between Italian and Spanish olive oils but also to define the Italian Protected Designation of Origin (PDO) Terra di Bari class, which turned out to be characterized by high-potential unique quality markers. The results obtained suggest that the combined platforms constitute a powerful tool to predict the authenticity and counterfeiting generated by deliberate and fraudulent mislabelling with respect to the geographical origin.
This may help the European National Competent Authorities (CAs) or the delegated Control and Certification Bodies (CBs) during official controls carried out to verify and ensure compliance with EU traceability and labeling requirements [1-4].

[1] Reg.(EC) No 178/2002 of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.
[2] Reg.(EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers.
[3] Commission Implementing Reg.(EU) No 29/2012 on marketing standards for olive oil.
[4] Reg.(EU) No 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs

Keywords: *Country-of-Origin Labelling (COOL), Fingerprinting, Ultra Performance Supercritical Fluid Chromatography (UPSFC), Fraud, Metabolomics*

L8*
HIGH CONTENT ANALYSIS: A SENSITIVE TOOL TO DETECT AND QUANTIFY THE CYTOTOXIC AND INTERACTIVE EFFECTS FOR SINGLE AND COMBINED CHEMICAL CONTAMINANTS PRESENT IN MAIZE

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Food safety is frequently compromised by the occurrence of chemical contaminants in food. Mycotoxins and heavy metals are ubiquitous in the environment, whilst the widespread use of pesticides in crop production contributes further to the chemical contamination of foods. Maize was used as a model crop to identify the severity in terms of human exposure when multiple contaminants are present. Maize (*Zea mays L.*) is one of the main cereals used in animal feed and as a human food source globally. Three mycotoxins, ochratoxin A (OTA), fumonisin B1 (FB1) and aflatoxin B1 (AFB1); two heavy metals, cadmium (Cd) and arsenic (As); and two organophosphate pesticides, chlorpyrifos (CP) and pirimiphos methyl (PM) are common contaminants of maize. High Content Analysis (HCA) measuring multi-parametric endpoints was used to determine cytotoxicity of complex mixtures of the listed mycotoxins, heavy metals and pesticides. Endpoints included nuclear intensity (NI), nuclear area (NA), plasma membrane permeability (PMP), mitochondrial membrane potential (MMP) and mitochondrial mass (MM). Contaminants were tested at their respective legal limits and above both individually and in mixtures. At concentrations representing legal limits of each individual contaminant in maize (3 ng/ml OTA, 1 µg/ml FB1, 2 ng/ml AFB1, 100 ng/ml Cd, 150 ng/ml As, 50 ng/ml CP and 5 µg/ml PM) mixtures (tertiary mycotoxins plus Cd/As) and (tertiary mycotoxins plus Cd/As/CP/PM) were cytotoxic for NA and MM endpoints with a difference of up to 13.6% ($p \leq 0.0001$) and 12% ($p \leq 0.0001$) respectively from control values. The most cytotoxic mixture was (tertiary mycotoxins plus Cd/As/CP/PM) across all 4 endpoints (NA, NI, MM and MMP) with increases up to 61.3%, 23.0%, 61.4% and 36.3% ($p \leq 0.0001$) respectively. Synergy was evident for two endpoints (NI and MM) at concentrations above legal limits, with differences between expected and measured values of 6.2 – 12.4% ($p \leq 0.05$) and 4.5–12.3% ($p \leq 0.001$) for NI and MM respectively. This study for the first time introduces a holistic approach to identify the impact in terms of toxicity to humans when multiple chemical contaminants are present in foodstuffs. Regulatory bodies must begin to contemplate how to safeguard the population when such mixtures of contaminants are found in foods. HCA is a highly novel and sensitive tool that could substantially help determine future regulatory limits, for single and combined toxins present in food, ensuring legislation is based on true risks to human health exposure.

Keywords: *high content analysis, mixture toxicity, synergy, food contaminants, cytotoxicity*

L9
FOOD FRAUD – OLD PROBLEMS NEW SOLUTIONS

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The integrity of our food supply is under intense scrutiny. Mislabelled meat, questions about the true origin of wines and spirits, concerns over the composition of herbs and spices, are some of the current issues that affect the food investigator and analyst today. They are not, however, new issues. The Romans often questioned the authenticity of the wine they drank; Victorian England was rife with highly adulterated and hazardous food, and horsemeat contamination of beef products was rife over 40 years ago. There has always been food fraud where ever there are two similar products with a significant price differential that the food analyst cannot distinguish between. What has changed is the technology that is now available to food scientists and analysts to verify the authenticity of the food we eat. Analytical methods and systems have a key role in food authentication as well as in identifying food fraud. The challenge to the scientific community is how latest developments in analytical chemistry and molecular biology can be used to help solve the problem. The latest strategies and technologies for food authentication will be presented including examples of the latest research priorities identified through the FoodIntegrity project (www.foodintegrity.eu). The potential of omic solutions will be discussed together with real examples of how the metabolome and microbiome can be exploited to help authenticate our food and identify fraud. Finally a brief summary of best practice in other sectors will be presented to inform the food sector on how best to assure the food supply.

Keywords: *food fraud, food authentication, omic technologies*

L10
FIGHTING FOOD FRAUD – WHEN ALL YOU HAVE IS A HAMMER, EVERYTHING LOOKS LIKE A NAIL: WHERE LABORATORY METHODS FAIL¶

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A significant challenge related to food misdescription in general, and food fraud in particular, is that many scientists who work in the field have extensive experience with specific methods and approaches, and they are mainly addressing the food fraud aspects that can be detected by the methods they already know. While extended application of existing methods and knowledge is a good thing, it becomes a problem when food fraud is more or less defined as that which can be detected by existing methods. Food fraud is not necessarily a food safety issue, and food fraud cannot necessarily be detected by the methods used in the food safety field. Many common food fraud issues may not have an analytical component at all; for instance when the fraudulent claim is related to original amount, weight or value, exact geographical origin, eco-label status, halal / kosher status, ethical production, or sustainable production. While some of these claims may have an analytical component, it is fairly obvious that other methods and approaches are needed in addition if the entirety of the claim is to be verified. This presentation outlines a more holistic approach to food fraud, where we start with the whole problem, and look at examples of food fraud that cannot be detected analytically, and what methods are needed for detection and prevention in those areas. The captured fish industry is a particularly relevant example here, as illegal, unreported and unregulated (IUU) fishing is a very real and quite extensive problem worldwide, and this necessarily leads to fraud when the fish with IUU origin enters the legal supply chain. This type of fraud is very difficult to detect at later stages in the supply chain (and impossible to detect analytically), but there are paper-trail based methods under development (mass balance accounting, input-output analysis) that can accompany analytical methods, and that can help detect and prevent fraud also in other food chains.

Keywords: *food fraud, traceability, mass balance accounting, input-output analysis*

L11
SPECTROSCOPIC BASED NON-TARGETED AUTHENTICATION OF PAPRIKA POWDER

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Securing the food chains from primary production to consumer ready food against any kind of contaminations and/or adulterations is a prerequisite for food safety. Therefore, partial aspects of the EU-project SPICED (Securing the Spices and Herbs Commodity Chains in Europe against Deliberate, Accidental or Natural Biological and Chemical Contamination, 7th framework program) are (i) to characterize the supply chains of spices and herbs in context with relevant chemical hazards that can lead to major natural, accidental or intentional contaminations, (ii) to ensure authenticity of spices and herbs by evaluation and improvement of non-targeted fingerprinting methods. The SPICED project inter alia aims at developing rapid and cost-efficient (high throughput) methodologies for the detection of natural, accidental and deliberate contaminations of spices and herbs with (unforeseen) chemical agents. For this purpose the application of e.g. nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared (FT–IR) spectroscopy for non-targeted analysis is evaluated using a representative set of authentic as well as adulterated spice and herb samples. In the presented study, authentic paprika powder samples (n=160; 32 different producers) were analysed by 1H NMR and FT–IR spectroscopy. The reliability of the measurements was confirmed by quality control sample. The acquired fingerprinting data were used to (i) identify an appropriate data pre-treatment/-processing for NMR and FT–IR data, respectively, (ii) explore the authentic data space by principal component analysis and (iii) to develop and evaluate various chemometric classification techniques (e.g. discriminant analysis) that allow distinguishing authentic from adulterated/contaminated samples, which were spiked with chromophoric substances, e.g. azo dyes or inorganic compounds.

Keywords: *food fingerprinting, adulteration, SPICED, authenticity, chemometrics*

L12*
THREE-DIMENSIONAL SEPARATION: A NOVEL STRATEGY FOR EFFECTIVE CLASSIFICATION OF SAFFRON ORIGIN

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Nowadays, interest in food authenticity has become a necessity for global food policies. Recently, advanced analytical methods, based on different separation techniques or stand alone have been coupled to high resolution mass spectrometry (HRMS), allowing food fingerprints to be achieved [1]. In this way, the development of analytical methods for the detection of adulteration and assessment of authenticity of food is vital; even more if the consumers are very willing to pay more for such products, such as saffron. In this research, a new strategy of saffron authentication based on metabolic fingerprinting was developed. In the first phase, a solid liquid extraction procedure was optimized, the aim was to isolate as maximal representation of small molecules contained in saffron as possible. Subsequently, attention was paid to an optimal setting of detection method for which UHPLC–TripleTOF 6600 (Sciex) was used. Afterwards, data processing and data pre-treatment was carried out in order to permit the identification of significant metabolites, which captured the bulk of variation between different datasets and may therefore potentially serve as biomarkers. The multivariate data analysis, a statistical comparisons and molecular feature identification, highlighted that glycerophospholipids and their oxidized lipids were significant markers according to their geographical origin. In a second step, a three-dimensional technique based on liquid chromatography, mass spectrometry and ion mobility was used for saffron authentication. Ion mobility (IM) is traditionally referred to ions traveling through a drift tube which has an applied electric field and a carrier buffer gas that opposes the ion motion. As a result, the migration time through the tube is characteristic of different ions, leading to the ability to distinguish different species. In this research, an unlike traditional ion mobility, called differential mobility separation (DMS) was used. In this case ions are not separated in time as they traverse the cell. They are separated in trajectory based on difference in their mobility between the high field and low field portions of the applied RF waveform (SV), also called separation voltage. At the same time, a second voltage, called compensation voltage (CoV), is used to correct the trajectory of the ion of interest which traverses the cell and into the orifice. In parallel, volatile reagents, such 2-propanol, were also introduced into the gas flow which chemically modifies how the ions interact with gas phase during the DMS separation, adding a new dimension to selectivity and dramatically increased separation capacity. This three-dimensional technique UHPLC–DMS–HRMS allowed differentiation of coeluting and unresolvable metabolites, as well as separation of stereoisomeric molecular ions based on their mobility. Therefore, a new strategy for effective classification of saffron origin was developed.

[1] Rubert J, Zachariášová M, Hajšlová J. Advances in high-resolution mass spectrometry based on metabolomics studies for food. Food Addit Contam Part A Chem Anal Control Expo Risk Assess.
In press 2015 http://dx.doi.org/10.1080/19440049.2015.1084539

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L13
FOOD AUTHENTICATION: CHALLENGES IN OFFICIAL CONTROL

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Modern analytical methods for verifying the authenticity as an integral component of food safety - Examples and developments from the proof of authenticity within food control. Due to precautionary aspects in consumer protection, methods are required in food and feed control which enable the verification of their declarations and specifications, i.e, a proof of authenticity, based on chemical/physical analyses of the respective item. In particular the increasing globalization of the food and also feed market requires reliable strategies to investigate the identity of goods with the aim of uncovering adulterated products, which repeatedly cause health risks to the consumer (e.g, the addition of melamine to milk products and feed materials). On the one hand changes/manipulations in relation to the authenticity of a food item (type, origin, production etc.), and on the other hand the identification of modifications which are critical for consumers' health (e.g, mixtures, additives), are of interest. Besides the classical analytical methods, measurements of stable isotopes but also the so-called profiling and fingerprinting-techniques have impor tant roles. The adaptation from research into routine applications takes actually place in some instances for the later techniques (e.g. NMR). Fingerprinting approaches offer enormous potential also in official control due to their typical abilities as high-through put and screening technique. Some examples will be presented and their possibilities and limits discussed. Particular emphasis will be put on open questions in view of the court-proof application such as exchangeability of data, validation and standardisation options.

Keywords: *food authenticity, profiling and fingerprinting techniques, exchangeability of data, validation and standardisation options*

L14
CRIMINALS ARE INVOLVED IN THE FOOD INDUSTRY – HOW DO WE CATCH THEM?

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"Criminals involved in class A drugs are becoming more involved in food crime/fraud because the penalties if caught are less severe" – Bart de Buck, Europol. To combat food crime the Food Safety Authority of Ireland (FSAI) has established a multi-agency Food Fraud Task Force (FFTF) comprising of scientists, regulators, police and customs officials. Without the input of scientists using the relevant and ever advancing analytical techniques fraud in Ireland regarding honey, fish, meat and alcohol would have gone undetected. Melissopalynology was used to identify the floral origins of honeys by identifying the pollens found in honey samples. DNA has and continues to be used in the speciation of chicken, fish, and beef to detect food fraud. We are working with major brand owners, who are sharing with our scientists unique product markers to detect counterfeit alcohol and prosecute those involved. Going forward stable isotopes or the microbiome may be used to determine the geographical origin of shellfish and thus help eliminate fraud in this industry. My presentation will describe how the FSAI and other agencies used their combined resources to identify food fraud and prosecute in the criminal courts those involved. I will outline how science and analytical techniques are proving successful in monitoring, detecting, deterring, disrupting and preventing food fraud. The ISO definition for food fraud is the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging; or false or misleading statements made about a product, for economic gain. Criminals in the pursuit of economic gain pay no regard to the protection of the health or interests of consumers. The absence of traceability for counterfeit food poses the greatest threat examples of which I will include in my presentation. Regulators, scientists, industry and the EU Commission are fighting back. I will present some of the emerging uses of science that are being used by the Irish authorities to tackle food fraud and the successes we have had to date. I will share the challenges that criminal prosecutions pose to scientists and regulators in the pursuit of conviction in the courts of justice for the perpetrators. The EU Commission are drafting a revised Regulation which will require Competent Authorities to include in their official controls identification of "violations perpetrated by fraudulent deceptive practices". We will need to be in a position through the use of science and investigative techniques to determine the nature and extent of these "fraudulent deceptive practices".¶

Keywords: *crime, fraud, deceptive, misleading*

L15
SCREENING OF PHOSPHODIESTERASE TYPE 5 INHIBITORS IN DIETARY SUPPLEMENTS USING LIQUID CHROMATOGRAPHY/ QUADRUPOLE–ORBITAL ION TRAP MASS SPECTROMETRY

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Increased availability of dietary supplements in the market and popularity among consumers have been accompanied by increased adulteration of these products with active pharmaceutical ingredients. Phosphodiesterase type 5 (PDE5) inhibitors and their unapproved designer analogues represent an important group of adulterants that have been frequently used to develop or intensify the desired biological effect in sexual performance supplements. Considering that PDE5 inhibitors can interact with certain prescription drugs and limited knowledge is available on safety and efficacy of the designer analogues, the presence of such compounds in dietary supplements may represent a serious health risk to consumers. This presentation will discuss development and validation of a liquid chromatography–high resolution mass spectrometry (LC–HRMS) method for screening and identification of PDE5 inhibitor drugs and their analogues in various types of dietary supplements. The data acquisition approach on a Q-Exactive Plus instrument combined full-scan MS, data dependent MS/MS and all ion fragmentation experiments to obtain comprehensive information in a non-targeted fashion. We will describe development of exact-mass product ion spectra database for about sixty PDE5 inhibitors, provide criteria for reliable analyte identification, and discuss targeted and non-targeted screening workflows for known and novel PDE5 inhibitors.

Keywords: *dietary supplements, phosphodiesterase type 5 inhibitors, adulteration, liquid chromatography-high resolution mass spectrometry*

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L16*
BIOMARKERS ENABELING DETECTION OF LINGONBERRIES (VACCINIUM VITIS-IDAEA) REPLACEMENT BY LESS VALUED CRANBERRIES (VACCINIUM MACROCARPON)

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Berry crops belonging to the *Vaccinium* genus represent a popular source of bioactive compounds, mainly polyphenols. The phytochemicals contained in lingonberries (*Vaccinium vitis-idaea*) have been shown to have a positive effect on cardiovascular and immune systems, some in vitro experiments have also documented anticancer activity. Another well-known effect of these red berries is prevention of urinary tract infections (UTI).
Recently, fraud suspicion on lingonberries-based products has been reported; *Vaccinium vitis-idaea* berries are partially or even totally replaced by less valued Cranberries (*Vaccinium macrocarpon*). Although PCR methods may provide solution, these are not available in routine laboratories focused on food analysis. On this account, alternative analytical strategies have to be searched. In this study, metabolomic fingerprinting employing instrumental platform consisting of ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (HPLC–HRMS/MS) has been investigated. For this purpose, polar (methanolic) and non-polar (hexane : isopropyl alcohol) extracts of 7 authentic varieties of *Vaccinium vitis-idaea* and 6 authentic varieties of *Vaccinium macrocarpon* together with their admixtures were prepared and analyzed by U-HPLC–HRMS/MS. Subsequently, chemometric evaluation was performed to assess the differences between the samples and to identify significant markers, which are present exclusively or more significantly in one of the fruit species. In parallel, accurate mass of ions in MS and MS/MS spectra, various software packages and online libraries were employed for tentative marker identification. The PCA (Principal Component Analysis) revealed significant differences between components in prepared extracts of lingonberries and cranberries. Clear clustering was found for both polar and non-polar extracts in positive and negative ionization modes. Especially, polyphenols (catechin, epicatechin, and procianidins) and glycosylated peonidins (peonidin 3-O-glucoside, peonidin pentose) were identified as markers responsible for lingonberries and cranberries differentiation.

Keywords: *cranberries, lingonberries, authenticity, metabolic fingerprinting, HRMS*

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L17*
MANUKA VERSUS KANUKA – DIFFERENTIATION OF NEW ZEALAND MONOFLORAL HONEYS BY MEANS OF CHEMOMETRICS

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The unique New Zealand manuka honey is characterized by a specific antibacterial activity towards other honeys which is caused by high amounts of methylglyoxal and also by further, so far unknown factors. Therefore, the valuable manuka honey is in great demand. This has led to more manuka honey being sold on the market than actually produced. Hence, only 1,700 t of manuka honey are produced in New Zealand each year. The honey actually sold as manuka honey worldwide is estimated at 10,000 t. In order to stop the ongoing fraud with the expensive honey, the New Zealand Government requires robust and practicable methods to define the monofloral manuka honey [1]. Hereby, the differentiation of manuka honey from the antibacterial, ineffective kanuka honey is of special interest. Due to identical pollen, a differentiation by pollen analysis is not possible. Furthermore, it is relatively easy to upgrade the kanuka honey to a high-quality manuka honey with methylglyo xal or its precursor dihydroxyacetone. In the last years, secondary plant metabolites such as phenolic acids, flavonoids, and norisoprenoids as well as volatile compounds were applied to prove the authenticity of monofloral honeys [2,3]. Pure monofloral manuka (n = 61) and kanuka (n = 16) honeys harvested in regions with monocultures were analyzed by means of HS–SPME–GC/MS. After comparing the aroma profiles, botanical marker compounds for manuka and kanuka honey were established using the principal component analysis (PCA). Finally, on the basis of these results, honeys obtained from the market (n = 45), which were declared as manuka honey, were investigated for their purity. Due to the data achieved, only 17 samples had originally been labeled correctly whereas the other labels were fraudulent. The results of this study will be presented.

[1] New Zealand Government. Interim Labelling Guide for Manuka Honey. July, 2014
[2] S. Trautvetter et al, Apidologie 2009, 40, 140-150
[3] S. Oelschlägel et al, Food Chemistry 2010, 64, 99

Keywords: *monofloral New Zealand honey, manuka honey, authentication, chemometrics, HS–SPME–GC/MS*

L18
RECENT ADVANCES IN GC-HIGH RESOLUTION MS FOR RESIDUE AND CONTAMINANT ANALYSIS IN FOOD

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Residue and contaminant analysis in food is a challenging task because of the high number (>1000) of substances potentially present. The golden standard in current routine residue analysis are targeted methods based on LC–ESI–MS/MS and GC–EI–MS/MS. Although these methods are highly sensitive and selective, and suited for multi-residue analysis, measurement of >200–300 analytes is less straightforward. LC and GC combined with full scan MS detection is more suited for this purpose and allows simultaneous measurement of an, in principle, unlimited number of analytes. The required selectivity can be obtained by using high resolution / high mass accuracy (Q)TOF and (Q)Orbitrap MS. While LC–HRMS has matured in the past 10 years and the technique can now be considered suited for routine application, developments in GC–EI–HRMS are more recent and have just started to catch up with LC. In this presentation the potential of GC–EI–high resolution MS will be illustrated using data obtained by GC–EI–Q-Orbitrap analysis. The effect of measurement at various resolving powers (15,000 to 120,000), and the use of mass extraction windows ranging from 100 ppm down to 5 ppm on selectivity is presented for extracts varying in complexity. A comparison to GC–EI–MS/MS is made. Sensitivity, quantitative performance and aspects related to identification and acceptability of the technique for analyses performed in a legislative framework will be discussed. Different strategies for library-based screening will be compared. An outlook will be given on how the developments in GC–HRMS may change residue analysis in the near future.

Keywords: *gas chromatography, high resolution mass spectrometry, pesticides, residue analysis, screening*

L19
PERFLUOROOCTANE SULFONATE (PFOS) DEPLETION IN BEEF CATTLE

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Perfluorooctane sulfonate (PFOS) is an industrial chemical that is used as a surfactant in several manufactured consumer products but is also a breakdown product from other chemical surfactants. As a result of its extensive use, PFOS is ubiquitous in the environment and is often detected in biosolids from waste water treatment plants. A common practice is the application of biosolids to pastures or croplands used for beef cattle feed production. The exposure of food animals to persistent organic chemicals has raised concerns about human exposure through the accumulation of PFOS in edible tissues. For these reasons, the United States Department of Agriculture (USDA) has undertaken a study to determine the absorption, distribution, and depletion of PFOS in beef cattle during a yearlong study period. Two Angus steers were given single oral bolus doses containing PFOS at 0.098 mg/kg body weight (bw) and four Angus heifers were given single bolus doses containing PFOS at 9.1 mg/kg bw. Two Angus steers served as undosed controls. Plasma was collected from each animal prior to, and at various intervals after dosing through 343 days. The high dose heifers were slaughtered at 105 days (n=2, ~1 PFOS half-life) and at 343 days (n=2, >2 PFOS half-lives), while all steers (2 dosed and 2 controls) were slaughtered at 343 days. Tissues collected included liver, kidney, backfat, intraperitoneal fat, muscle (ribeye, tenderloin, rump, and shoulder), bone, and skin (only from animals at 343 days). PFOS residues were extracted by liquid-liquid or liquid-solid ion pairing extraction methods and quantified by liquid chromatography-quadrupole time of flight mass spectrometry. The average maximum concentrations of PFOS in plasma for the steers and heifers were 0.61 µg/mL (n=2) and 65±12 µg/mL (n=4), respectively. Average plasma elimination half-lives for steers and heifers were estimated to be 115 days and 119 days, respectively. Apart from plasma, liver contained the highest tissue concentrations for steers at 149.0 ng/g wet weight (ww). Liver concentrations from the heifers at 105 days and 343 days were 8,765.3 ng/g ww and 4,744.1 ng/g ww, respectively. The second highest PFOS tissue concentrations were in the kidney for steers at 81.9 ng/g ww and for heifers at 3,964.3 ng/g ww (105 days) and 2,356.6 ng/g ww (343 days). For edible tissues such as muscle (tenderloin, shoulder, ribeye, and rump), the range of PFOS concentrations for steers was 4.1–7.2 ng/g and for heifers were 364.1–1,196.8 ng/g ww (105 days) and 168.7-394.0 ng/g ww (343 days). At 343 days PFOS residues were still detectable (limit of detection was ~2 ng/g in tissue) in all tissues tested including fat and muscle indicating there is potential for human exposure to PFOS even after an extended withdrawal period.

Keywords: *perfluorooctane sulfonate, beef cattle, plasma elimination half-life, absorption, distribution*

L20
ANALYTICAL STRATEGY BASED ON ISOTOPIC CLUSTER IDENTIFICATION AND MASS DEFECT TO HIGHLIGHT HALOGENATED ENVIRONMENTAL CONTAMINANTS

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In the past decades, many Persistent Organic Pollutants (POPs) have become ubiquitous. Increasing regulatory pressure against them, ultimately thought the Stockholm Convention, has supported the emergence of even more alternatives which might also act as environmental contaminants. For the characterised substances, the analytical chemist, in charge of diet exposure or body burden studies, usually develops suitable targeted approaches based on mass spectrometry (MS). These methods are quantitative, based on the isotopic dilution principle whenever possible. Limited to this approach, one will not observe the other substances (e.g. degradation products, emerging compounds). Identifying these substances we are exposed to in a sample requires another approach, untargeted. In parallel, recent generations of high resolution MS (HRMS) instruments (e.g. orbital trap, TOF) coupled to chromatographic systems, allowing for acquiring full scan footprints, tend to general ise in laboratories. We suggest an untargeted approach to identify environmental contaminants (or related compounds) in complex biological samples. We use chlorine (Cl) and bromine (Br) specificities (mass defect, isotopic clusters) as common feature to most of the lipophilic POPs. The workflow can be divided in 5 steps: (1) sample preparation, (2) data acquisition in HRMS, (3) automatic integration of peaks, (4) automatic Cl and Br isotopic clusters identification and (5) interpretation. The sample preparation must be short and poorly selective (fat removal only). In the present work, we only applied a liquid-liquid partitioning between concentrated sulphuric acid and fat extracted from fatty samples. We selected LC–ESI–HRMS for data acquisition operating at a resolution of 70,000 at m/z 200 (Q-Exactive, Thermo). The automatic integration was achieved using the centWave function of xcms package in the "R" environment. This peak picking can generate up to thousands of features characterised by a m/z, a retention time and an intensity. Then we developed an Excel® VBA macro to identify the Cl and Br isotopic clusters. The interpretation of identified clusters was made more comfortable using an Akin-plot graphical representation and VBA macro tool. The workflow was tested on an eel sample. Among the 15,250 features obtained after the peak picking step, 3,920 were grouped in 1,283 clusters. Chlorinated paraffin series, from C10 to C33 and containing 5 to 10 chlorine atoms were tentatively identified. Among others, hydroxylated PCBs and PBDEs, pesticides and polyhalogenated phenolic compounds were also tentatively identified. The strategy was also applied to samples collected in the frame of animal experiments to identify (i) alpha-HBCDD metabolites (laying hen, broiler, pork) and (ii) chlormadinone metabolites (calf). Then, the potential of such strategy appeared promising. A future work will be to increase automation in interpretation step, by incrementing a data base for example.

Keywords: *persistent organic pollutant, mass defect, akin plot, isotopic cluster, screening*

L21
ESTABLISHMENT OF A HIGH-THROUGHPUT DETECTION SYSTEM FOR ENDOCRINE DISRUPTING CHEMICALS IN FOOD

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Endocrine disrupting chemicals (EDCs) in food arise either from natural or anthropogenic origin. Plasticizers and other plastic additives from food packaging or pestizides as residues on fruits and vegetables belong to the most focused substances within these chemicals or mixtures. There is still a lack of high-throughput platforms for identification and characterization of EDC related hazards in food. Existing analyzing platforms for endocrine activity are mostly insufficient because they do not reflect the complex interactions of bioactive chemicals and hormones. Therefor we aim at developing a microfluidic device that combines a cell- and protein- microarray allowing the rapid analysis of specific EDC´s, their toxicity and bioavailability in food samples. The protein chip quantifies 13 biomarkers related to various endpoints in a sandwich immunoassay format in the cell culture supernatant of EDC exposed MCF-7 cells cultured in serum free media. The human breast adenocarcinoma cell line MCF-7 is hormone sensitive and retained a number of properties expressed by breast epithelium in vivo and features receptors for estrogen, androgen, progesterone, glucocorticoid, insulin and L-3, 3´, 5-triiodothyronine. Estrogen, specifically estradiol and even estrogen active substances are capable of modulating the expression and secretion of insulin-like growth factor binding proteins (IGFBPs), insulin-like growth factor (IGF)-1, vascular epidermal growth factor (VEGF), interleukin (IL)-8, matrix metallopeptidase (MMP)-9, estrogen-induced pS2 and endotstatin. It was reported that the proteins IGF-1, IGFBP-3 and endostatin decreased after estradiol exposure while IGFBP-4, VEGF, pS2, MMP-9 showed an increased protein secretion. The addition of the antiestrogen tamoxifen opposed these effects. Furthermore the expression of e.g. IGFBP-4 is also positively correlated with estrogen receptor (ER) status in mammary tumors. Steroid hormones like estradiol promote the development and maintenance of primary and secondary female reproductive tissues. After estrogen exposure MCF-7 cells proliferate. Including this effect the detection of the estrogen regulated proteins and the parallel measurement of the proliferation of the cells will be part of the whole system. In ongoing experiments biomarker secretion following exposure to endogen hormones (as control for an ER agonists), EDC´s and ER receptor antagonists like tamoxifen is compared and used for validation of the biomarker chip.

Keywords: *EDC, food contaminants, detection system, microfluidics, hormones*

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L22*
THE USE OF A MULTIPLEXING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRIC SYSTEM FOR INCREASED THROUGHPUT OF SAMPLES FOR VETERINARY DRUG RESIDUE ANALYSES

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Laboratories responsible for food safety are being pressured to increase throughput and reduce cost of analyses. The focus of this work is on the analytical advantages of using a multiplexing liquid chromatography system, coupled with mass spectrometry, for increased sample throughput on one mass spectrometric system. Examples of these advantages include use of different mobile phases, columns, polarity, and/or flow rates for each system. As only the target analytes are diverted to the electrospray source, heart cutting is an incorporated component of the injection cycle. Heart cutting, coupled with clean extracts using new bench methodologies, minimizes instrument contamination and thereby increases the time required between maintenance periods. Importantly, the increased number of sample runs per hour allows for more quality control (QC), thus increasing the confidence of reported results. Two case studies of single lab validation data will be present ed in detail, which demonstrate the use of multiplexing; one examining coccidiostats in animal tissues, and one examining parasiticides and antibiotics in raw milk of bovine and caprine.

Keywords: *multiplexing, liquid chromatography, mass spectrometry, veterinary drugs, high throughput*

L23
VIBRATIONAL SPECTROSCOPY TECHNIQUES ARE SUITABLE FOR REPRESENTATIVE AND UNTARGETED ANALYSIS OF FOOD AND FEED PRODUCTS

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Vibrational techniques are key tools for risk managers seeking to tackle the source of problems at the entrance of food/feed chains. For food inspection bodies, these rapid techniques enable them to obtain analytical results from a large number of samples and for multiple parameters simultaneously. They could help to prevent unfair commercial practices and therefore ensure effective consumer protection, especially during food/feed crises. These analytical techniques have been largely used in the last years proving their performances in different sectors by being very precise and validated according to international standards. Vibrational techniques, as Near Infrared and Raman spectroscopy, are inevitably linked to data treatment or chemometric methods. By using the adequate vibrational technology and chemometric strategy, it is possible to take advantage of the heterogeneity of a sample in order to, for instance, perform an efficient detection of contaminants and to characterize a certain product. Recent advances allow in-situ analysis using, for instance, Raman, IR microscopy or NIR hyperspectral imaging, techniques that add a spatial dimension to the spectra. Using such instruments, up to several thousand of spectra per sample can be simultaneously collected. They are gathered in order to generate a hypercube that includes the wavelengths, the absorbance values and the spatial information. In this work, different examples in the food and feed sectors will be presented to demonstrate that the combination of the right sampling procedure with the adequate vibrational spectroscopic technique and chemometrics allows detecting contaminants at low level in a targeted and untargeted way.

Keywords: *infrared, Raman, sampling, contaminants, untargeted detection*

L24*
IN SITU DETECTION OF FUNGICIDE ON FRUIT'S PEAL BY SURFACE ENHANCED RAMAN SCATTERING

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In the last decades, Surface-Enhanced Raman Scattering (SERS) has become a mature vibrational spectroscopic technique and the number of applications in the chemical, material, and life sciences has rapidly increased [1]. In comparison with the most diffused techniques such as high performance liquid chromatography (HPLC) and mass-spectrometry, Raman spectroscopy allows fast detection times, high selectivity due to the Raman fingerprint of molecules without any preliminary treatment of the sample. Moreover, the sensitivity of the traditional Raman technique can be increased of several orders of magnitude in SERS technique due to the enhancement of the Raman scattering of molecules absorbed onto, or microscopically close to, a suitable plasmonically active surface, such as roughened nanostructured metal surfaces, or metal colloids [2]. For all these reasons SERS represents a good candidate for food control analysis. Nevertheless, standardized methods of produc tion and application of SERS systems are still needed in order to provide reproducible analytical methods, especially for in-situ applications in food analysis [3]. For this study pyrimethanil fungicide was chosen as a representative test material. Pyrimethanil is an active ingredient of a commonly used pesticide, which is employed on several horticultural species. The scope of this research is to provide a reproducible SERS procedure to analyze fungicide's traces directly on fruit by depositing NPs on its surface. This represents an innovative approach with respect to the employment of typical SERS substrates, which requires extraction and transfer of the analyte from the matrix to an active surface. In this framework, four types of gold nanoparticles (AuNPs) i.e. spherical AuNPs with 50 and 120 nm diameter, nano-rods and silica covered AuNPs were prepared by simple protocols [4; 5]. In order to ensure greater reliability and accuracy of SERS analysis, gold NPs with diffe rent size and shape were first characterized using different techniques (UV-Vis spectroscopy, Scanning Electron Microscopy, Dynamic Light Scattering and Raman). A comparative study of SERS efficiency for fungicide detection on a model system between different AuNPs will be presented. Moreover, an example of SERS analysis of fungicide traces on an apple's peal will also be shown.

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Keywords: *surface enhanced Raman scattering, gold nanoparticles, in situ analysis, fruit control, fungicide*

L25
TRAVELING–WAVE ION MOBILITY PROVIDES ADDITIONAL CONFIDENCE IN THE INTERPRETATION OF GROWTH PROMOTERS CHEMICAL SIGNALS

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Despite the potential risk for animal welfare and public health issue related to the chemical safety of animal products, some farmers – under pressure due to the increase number of consumers and the competitiveness of food markets - may choose to improve their production using growth promoting factors. While efficient analytical strategies are currently implemented at the European level to ensure safe food to the consumers, some analytical challenges still remain to be overcome, in particular when novel drugs or cocktails are used. Therefore, even more specific and sensitive protocols are required to tackle this issue. While the coupling of travelling wave ion mobility spectrometry (TWIMS) and mass spectrometry is mainly reported for structural purposes, we studied its potential in enhancing compounds analysis such as growth promoters used in livestock animals at trace concentrations. TWIM separation provides a 3D separation when associated to UPLC and HRMS. Ions are then differentiated according to their retention time, their mass-to-charge ratio and their mobility time related to their spatial arrangement (=CCS). The CCS could therefore be used as new identification point for analyte confirmation purposes and provide additional confidence in the assignment of chemical species. The orthogonal mode of separation enables clean-up of spectral information for better both selectivity and sensitivity of the detection. Applications to current challenges in the field (separation of βb-agonists isomers in various biological matrices (retina, urine, muscle), detection of somatotropin in milk, identification of SARMs in urine) are presented and discussed.

Keywords: *IMS, food safety, specificity, drift time, CCS*

L26
ENDOGENOUS ORIGIN OF PREDNISOLONE: A REVIEW

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Prednisolone is a synthetic glucocorticoid involved in carbohydrate and protein metabolism. Due to its anti-inflammatory, anti-allergic and immunosuppressive properties, prednisolone is widely used for human and livestock therapeutic purposes. However, regarding human and animal doping, as well as animal breeding, its use is strictly regulated. According to the World Anti-Doping Agency (WADA) Code, the systemic administration (oral or by injection) of corticosteroids is forbidden in competition but allowed when medically necessary and when applied in non-systemic way (dermatological, inhaler and intra-articular administration); the Minimum Required Performance Limit (MRPL) has therefore been set at 30 ng/ml. Prednisolone is also classified by the Fédération Equestre Internationale (FEI) as forbidden substance for racing horses and can only be used for a therapeutic purpose (treatment of existing inflammatory conditions related to illness or injury). It can not be administered 24 hours prior to competition and its administration seven days prior to a competition should be reported. In Europe, prednisolone is not allowed in animal breeding as growth-promoting agent and Maximum Residue Limits (MRLs) have been set for prednisolone in edible tissues of bovine and equidae (Directive 2010/37/CE). Monitoring of prednisolone in livestock urine is included in National Control Plans of European Member States. Due to the improvement of the performance of analytical methods, prednisolone residues have recently been found at low levels in bovine and porcine urine samples without any direct evidence of unauthorized administration. Hypotheses have been proposed to explain the natural occurrence of prednisolone, among which a relation with stress and the conversion of cortisol to prednisolone by dehydrogenase activity. Several studies have been performed on bovine and porcine to elucidate the presence of prednisolone at low levels in urine. Data are available concerning natural concentrations of prednisolone in bovine, porcine, equine and human urine. Taking into account the endogenous origin of prednisolone, analytical strategies should be developed to avoid false non-compliant results in both doping control and control of food producing animals; this includes setting of action limits, identification of biomarkers, metabolomics and/or use of isotope-ratio mass spectrometry. The aim of this presentation is to review the results of human and animal studies investigating the endogenous origin of prednisolone, including observed levels in non-treated humans/animals, possible formation, relation with stress and analytical strategies that could be applied to discriminate between endogenous and exogenous origins.

Keywords: *prednisolone, endogenous, urine, livestock, human*

L27
SOLID-STATE ARSENIC SPECIATION IN FOODSTUFF PRODUCTS BY ETV–ICP OES

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Direct solid state analysis is becoming more and more popular as it reduces sample preparation, contamination risks and losses of volatile elements. Electrothermal vaporization (ETV) coupled with ICP OES allows direct solid sampling analysis. But even if several applications of this coupling have been reported in different samples (biological, environmental…) for total trace elements determination, applications related to speciation analysis are very scarce. Arsenic is one the most important elements addressed in terms of speciation. Indeed, the single determination of total As may lead to an overestimation of the toxicological impact as big differences in terms of toxicity exist between inorganic and organic arsenocompounds. The presentation will therefore focus on the developments performed to achieve arsenic speciation in different foodstuff products (rice, sea products…) by ETV–ICP OES. Special attention will be paid on the achievement of quantitative data (that still remain a critical point for direct solid state analysis) and the comparison with data obtained by classical HPLC–ICPMS analysis.

Keywords: *ETV–ICP OES, As speciation*

L28
GENERAL INTRODUCTION INTO THE TASKS OF EURLS

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L29
EXPERIENCES, ACHIEVEMENTS AND CHALLENGES OF JRC HOSTED EU REFERENCE LABORATORIES FOR CONTAMINANTS

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Since almost a decade the Institute for Reference Materials and Measurements hosts 3 European Reference Laboratories (EURLs) in the field of food and feed contaminants. The specific targets of these EURLs are heavy metals, mycotoxins and poly aromatic hydrocarbons based on a legal mandate in EU legislation. Aim of the EURL activities is to ensure a high level of measurement capacity in European Union Member States' control laboratories through a network of National Reference Laboratories (NRLs). The mandate of all EURLs and NRLs is laid down in Regulation (EC) No 882/2004 of the European Parliament and of the Council.
Key pillars in maintaining a high measurement capacity within European control laboratories are, among others, comparative testing schemes organised by each EURL in regular frequency each year as well as the drafting of guidance documents for and within the Network of Reference Laboratories.

This presentation will give specific examples what challenges the EURLs encountered during their duty, the experience gained thereof and the resulting achievements for the benefit of strengthening official control for food and feed safety in this sector.

Keywords: *heavy metals, mycotoxins, polyaromatic hydrocarbons, comparative testing schemes and guidance documents for NRLs, official control for food and feed safety*

L30
CHALLENGES AND ACHIEVEMENTS OF THE EU REFERENCE LABORATORY FOR DIOXINS AND PCBS

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The networks of the EU–RLs and the National Reference Laboratories (NRLs) should contribute to a high quality and uniformity of analytical results. In the field of dioxins and PCBs, for this purpose harmonized analytical criteria for methods for control of feed and food were last amended in 2014. Major scientific amendments comprise criteria for use of GC–MS/MS as confirmatory method. The revised chapter "Field of Application" clarifies the goals and limitations of screening methods and defines confirmatory methods (based on GC–MS/MS or GC–HRMS) for confirmation of compliance or non-compliance and for other purposes such as determination of low background levels, following of time trends or exposure assessment (based only on GC–HRMS). The comprehensive validation of CALUX-based screening methods in the requested close cooperation with a laboratory applying confirmatory methods is presented separately. Self-control of industry is an important pillar for safety of feed and food. The question was raised if accreditation according to the EN 17025 standard is sufficient to guarantee a high quality of the results or if the Commission Regulations are binding also for self-control of industry. In contrast to EN 17025, Commission Regulations (EU) 589/2014 (for food) and 709/2014 (for feed) prescribe parameters to be checked plus detailed specifications. Comparability and reliability of results of methods for determination of dioxins, dioxin-like PCBs and non-dioxin-like-PCBs in feed and food are, therefore, only ensured if the specific requirements of the "lex specialis" are met. The Core Working Group "Measurement Uncertainty" of the EU-RL/NRL-network is preparing a Guidance Document "Evaluation of Measurement Uncertainty for Contaminants, in particular PCDD/Fs and PCBs Analysis, using Mass Spectrometry Isotope Dilution Technique". In cooperation with the other three EU–RLs for contaminants (PAHs, Heavy Metals and Mycotoxins), a joint document "Guide on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" is being prepared. An important pillar for quality control is the performance of PT studies. The EU–RL for Dioxins and PCBs is accredited by the Deutsche Akkreditierungsstelle (DAkkS) under the terms of DIN EN ISO/IEC 17043:2010 to carry out proficiency testing/ interlaboratory comparisons in the testing field of chemical analysis and bioanalytical methods for determination of PCDD/Fs and PCBs in food and feed. An overview on the two PTs performed annually by the EU–RL is presented separately. The PTs comprise also a study on determination of brominated flame retardants in cod liver and fish liver oil. With two workshops organised annually for discussion of PTs and other issues, the EU–RL/NRL network has developed a close and fruitful cooperation.

Keywords: *EU reference laboratory, dioxins, PCB*

Acknowledgement: *We would like to thank the EU Commission for all support and the network of EU–RL and National Reference Laboratories (NRLs) for the scientific contributions.*

L31
LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY WITHIN PESTICIDE RESIDUE CONTROL IN FOOD

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LC and GC–HRMS, are destined to play a central role in routine pesticide residue analysis. These modern instruments can compete in performance with triple quads, giving additional important advantages as a consequence of their unmatched selectivity and full scan information enabling easier and faster workflow within the laboratory. However HRMS has not reached at the moment a deep penetration as the QqQ technology in pesticide residue laboratories. There are a number of reasons why the QqQ instruments are considered more reliable and rugged. But, most of these analytical drawbacks in favor to QqQ, especially in sensitivity and dynamic range have been equilibrated with the updated instruments. The use of HRMS full scan data for quantitative use will certainly become the standard. Q–HRMS instruments (e.g. Q–TOF, Q–Orbitrap) are clearly more capable than single stage HRMS allowing a more confident identification by combining full scan and HRMS/MS information by using data dependent or independent modes. The aim of this work is to present various HRMS practical approaches using different QTOF and Orbitrap MS/MS platforms for qual and quant evaluation of residues in fruits and vegetables. The evaluation of the advantages and drawbacks can demonstrate the potential application of these MS platforms in the routine work flow of the pesticide residue laboratories. Additionally regulatory aspects about method validation that covers the development and validation of the HRMS methods are also evaluated.

Keywords: *pesticides, mass spectrometry, LC–Q–TOF, LC–Q–Orbitrap*

L32
RESIDUE CONTROL – CONTRIBUTION OF EURLS TO IMPROVEMENT OF QUALITY, EFFICIENCY AND HARMONISATION

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The LC–MS/MS technique was in its infancy when the EU system for residue control of veterinary drugs was established. Analyses were carried out by GC–MS, HPLC–DAD and HPLC–FLU. The scope of the methods covered single or very few analytes of one substance group. The evolution of LC–MS/MS instruments boosted the sensitivity and robustness, and with that also the development of multi-methods. Beginning in 2000, the EURL launched the development and promotion of multi-methods, first for ß-agonists and afterwards, step by step, for the other substance groups in its field of responsibility. Today we do not only talk about effective multi-methods within one substance group, but about substance-group-comprehensive methods. The extension and improvement of a method to new analytes depends on the availability of the corresponding standard compounds and stable isotopically labelled internal standards. The EURL promoted the synthesis of standards and internal standards at a very early stage. Another important part of the EURL work is the realisation of animal studies in order to produce incurred material as similar as possible to real-life samples for the performance of proficiency tests. Moreover incurred materials represent a valuable tool in the search for new metabolites and conjugated residues, in the investigation of analyte stability in matrix as well as in the optimisation of sampling, which is important in particular for banned or non-authorised compounds. The evaluation of the PT-results of the last 10 years shows a clear improvement of methods with respect to performance and number of analytes, which is also reflected in the development of the NRCPs of the EU Member States. The third important part of the EURL work is the development and implementation of validation approaches like the matrix-comprehensive in-house validation concept based on an experimental design, which has expanded into the European legislation.

Keywords: *veterinary drugs residues control, proficiency testing, development and implementation of validation approaches*

L33
TECHNICAL CHALLENGES IN FOOD SAFETY AND INNOVATION FOR FOOD CONTACT MATERIALS: THE ROLE THE JRC AS SUPPORT TO EU POLICIES AND EURL

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The importance food contact materials is recognised as key in achieving long lasting quality and shelf life. These materials include food processing, packaging as well as kitchen appliances, cutlery. Yet, while new materials and technologies are constantly being developed to great benefits for the consumer, these must also be safe. This safety relies on ensuring that no release of chemical substances might occur in unsafe levels from materials involved in the food processing chain, packaging materials or kitchenware, into the food. Materials and articles in contact with foods fall under a specific legislation at the EU level to ensure the safety of the consumer while facilitating trade. Specific limits are imposed on potential release of substances during contact with foods. The development of new harmonised legislation and functioning rules is based with science as a driver at its core. Considering the increasing importance of this sector in the food chain, the presentation will illustrate long standing role of JRC as scientifically underpinning entity in science for policy support since 1995 on FCM as well as in its role of EU reference Laboratory (EURL–FCM) since 2004. The EURL–FCM has developed databases of more than 450 substances and 300+ methods for regulated FCM substances for compliance and enforceability. In 2008–2014 the EURL–FCM organised more than 30 proficiency exercises, representing more than 80 matrix-measurands combinations, resulting in increased performance in official controls. Close to 20 standard tests materials were developed and produced. It also organised trainings to improve the performance of the official controls in the EU. The activities supported a wide range of issues from gaskets, dry foods, packaging inks to kitchenware, tableware. The JRC also developed guidelines for sampling and testing to improve cost-effectiveness and robustness of laboratory tests. These guidelines are now recognised worldwide and have provided a solid basis for the harmonised enforcement of the legislation. As a result non-compliance has decreased significantly, for example from 11% to 2% on melamine and polyamide imported kitchenware, thus smoothing the international trade. This role also enabled a steady increase in performance of the laboratories for official controls across the EU. In its policy support capacity, the JRC organised more than 30 fora with stakeholders to contribute with a sound scientific basis to global safety for food contact materials. It completed research on the market landscape of baby bottles anticipating policy needs on potential future safety issues. It supported the work of the European Food Safety Agency (EFSA) in 2 exposure assessments and contributed to 2 major RTD projects related to food contact on interactions between food and packages which led to changes of legislation and development of new harmonisation. Examples of the most relevant impact will be highlighted.

Keywords: *food contact, food safety, packaging, kitchenware, contaminants*

L34
ANALYTICAL CHALLENGES IN THE TRANSITION FROM BIOLOGICAL TO CHEMICAL METHODS FOR THE CONTROL OF MARINE BIOTOXINS IN SEAFOOD

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Marine Biotoxins are natural contaminants present in the marine environment due to the proliferations of toxic microalgae. They accumulate in seafood mostly in bivalve mollusks. The toxicity associated to the consumption of these contaminated seafood can be different depending on the toxins involved in the contamination, although the most frequent, which are regulated in the EU are Lipophilic shellfish toxins (LPTs), Paralytic shellfish toxins (PSTs) and Amnesic shellfish toxins (ASPs). The transition from animal tests (Mouse Bioassay) to physic-chemical methods such as Liquid Chromatography coupled to different detection modes (UV, FLD and MS/MS), has been challenging, nevertheless methodological improvements carried out over the last few years, allowed to obtain successful results which have contributed to expand the knowledge in the field. The complexity of the matrices as well as the nature of the analyte have been critical issues to be faced and also the fact of transitioning from a biological approach where total toxicity has been taken into account, to a quantitative method where well established performance criteria have to be evaluated, made this transition more challenging but on the other hand the application of validated approaches has contributed to the methodological harmonization. The work presented here provides an update of the situation of the control of marine biotoxins in the EU. Analytical issues of particular interest when applying quantitative methods and in particular multitoxin methods are going to be discussed and the present and future needs will be also explored. An updated situation of the methodological advances for the control of emerging marine biotoxins in the EU will be also discussed.

Keywords: *marine biotoxins, lipophilic toxins, paralytic toxins, HPLC- mass spectrometry, fluorescence*

L35
IDENTIFICATION AND PRIORITIZATION OF CHEMICAL HAZARDS IN ANIMAL PRODUCTS MONITORED BY THE U. S. NATIONAL RESIDUE PROGRAM

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The U. S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) is responsible for ensuring that the U.S. commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged. Under the U. S. National Residue Program (NRP), FSIS monitors these products for the presence of veterinary drugs, pesticides and environmental contaminants based on tolerances set by the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA). In 2012, adoption of multi-residue methods (MRMs) permitted FSIS to expand the number of monitored chemicals. The NRP requires the cooperation and collaboration of sister agencies (FDA, EPA and Centers for Disease Control and Prevention (CDC) for its successful design and implementation. The NRP annual sampling plan is based on:
1) prior NRP findings of chemical residues in meat, poultry, and egg products,
2) FDA veterinary drug inventories comple ted during on-farm visits and investigation information, and
3) pesticides and environmental contaminants of current concern to the EPA.
FSIS is currently developing a model to identify and prioritize chemicals of public health concern that may be present in FSIS regulated products but are not included in current NRP process. This model incorporates chemical properties and public health effects in the prioritization algorithm.

Keywords: *pesticides, meat, poultry, interagency, multi-residue*

L36
ANALYTICAL CHALLENGES FOR FOOD SAFETY AND COMPLIANCE: INDUSTRIAL PERSPECTIVE

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The management of food safety and compliance today requires an integrated approach and close collaboration of all the teams along the production chain “from farm to fork”. This includes agricultural services on the field, regulatory and quality management at local and global level and analytical experts that develop and implement the harmonized method portfolio with the right tool for the right application at the right location. Even with all these elements well implemented there are several reasons to expect new issues in the food chain: growing complexity of global food supply and production, longer and more complex supply chains, development of scientific knowledge, growing food demand, climate change, differences between risk perception and scientific assessment etc. Therefore current challenges addressed are: fast detection and response to a new threat/issue, method alignment whenever possible with internationally recognized standards to avoid trade disputes, upstream testing with faster, cheaper and broader screening methods, agreed performance criteria for rapid tests, higher throughput methods and automation, multi-residue analytical methods with generic sample preparation and transversal standards covering many different commodities or matrices, etc. Some of the above challenges will be illustrated by business case studies

Keywords*: chemical contaminants, adulteration, analysis, challenges*

Acknowledgement: *R. Stadler, T. Delatour, P. Zbinden*

L37
CURRENT APPROACHES FOR MANAGING FOOD CONTACT MATERIALS ISSUES: THE PRIMARY AROMATIC AMINES CASE

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Primary Aromatic Amines (PAAs) are toxic compounds and suspected human carcinogens. One of the sources of PAAs into food is the polyurethane-based adhesives employed for the manufacture of multilayer films for food packaging. PAAs are also often used as starting compounds in the production of azo dyes, so traces can remain in the finished pigments and possibly migrate into foodstuffs from printed food packaging. Another possible source of aromatic amines might be the packaging made of recycled paper due to the massive use of printed paper in the recycling process. PAAs migration into foodstuff is subjected to restrictions according to European Union Regulation 10/2011: plastic material and articles shall not release primary aromatic amines in detectable quantity and the detection limit is set to 0.01 mg of substances per kg of food or food simulant and it applies to the sum of primary aromatic amines released. Currently the accepted standard approach for the analysis of PAAs is a spectrophotometric method: although good sensitivity, the lack of selectivity and the high risk of false positive results have required the development of alternative analytical methods. A first goal of our research work was the development and validation of a LC-Orbitrap–full scan-HRMS for the determination of migration levels of 22 PAAs in food simulants. A very fast and efficient separation (<11 min) of PAAs was achieved, combined with direct injection of the simulants without any pre-treatment step and a very high sensitivity well below μg kg−1 level in almost all the investigated compounds. In addition, the approach based on full scan-HRMS detection opens the way to a comprehensive analysis and to the possibility to perform “retrospective” screening. At the same time, the expensive equipment and the need of high-degree specialized operators can often represent a drawback in a standard packaging control laboratory. Taking into account the increasing availability of relatively inexpensive LC–MS instrumentation, in parallel its applicability for potential routine analysis was strengthened as a second goal: a reliable LC–MS analytical method that allows the selective quantitation of the most relevant PAAs was developed. Suitability of this cost-effective alternative method was demonstrated for a wide range of commercial samples, chosen among different producers of the most common used food packaging plastic and paperboard categories and then analyzed to assess the risk related to PAAs migration. Finally, this method turned out to be very useful to monitor the evolution of potential PAAs migration during the industrial curing process of multilayer packaging films, prior to their release for delivery to the food industry end user.

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[2] F. Lambertini, V. Di Lallo, D. Catellani, M. Mattarozzi, M. Careri, M. Suman (2014) Journal of Mass Spectrometry 49, 870

Keywords: *primary aromatic amines, food packaging materials, liquid chromatography mass spectrometry, high resolution mass spectrometry, industrial curing adhesives*

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L38
FOOD FRAUD ANTICIPATION: DEVELOPMENT OF A QUICK AND SIMPLE QUANTITATIVE MULTI-COMPOUND METHOD FOR THE DETECTION OF NITROGENOUS COMPOUNDS IN ROUTINE

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Over the last years food fraud has become concern for the food supply chain. Several food fraud cases involving high-nitrogen content chemicals were observed raising safety and compliance issues. Such compounds can be added into food raw materials increasing artificially their protein content for economically-motivated adulteration. Several analytical methods for nitrogenous compound detection have been published. They cover a large number of compounds but provide qualitative results with high limits of detection. This gives the possibility to evidence food fraud adulteration but not to properly estimate contamination levels, e.g. in finished products. On the other hand, a number of recent reports have described quantitative methods able to reach lower limits of quantification, but covering only a limited range of compounds. In addition, the sample preparation is often time consuming and not fit for high throughput routine analysis. Our study reports a fast and simple multi-compound method capable to detect high levels of nitrogen-rich compounds based on adulteration but also low levels due to contamination. The presentation will review the developed multi-compound method covering fourteen nitrogenous compounds, including the big four which are melamine, ammeline, ammelide and cyanuric acid. This method is using a simple and fast sample preparation based on dilution and clean-up by dispersive solid phase extraction. After separation with hydrophilic interaction chromatography, detection is done in one single run by liquid-chromatography tandem mass-spectrometry using positive/negative switching. Quantification is carried out by isotopic dilution reaching limits of quantification between 0.05 mg/kg and 0.20 mg/kg. Validation results, established according to SANCO 12571/2013, will be presented. To ensure the method performance/ruggedness analytical standards from different suppliers have been tested for their purity and a 6-month stability test of all diluted compounds has been performed. The obtained results will be shown and discussed.

Keywords: *nitrogenous compounds, food fraud anticipation, liquid-chromatography tandem mass-spectrometry*

L39*
IDENTIFICATION OF DEGRADATION PRODUCTS ORIGINATED FROM 3-MONOCHLORO-1,2-PROPANEDIOL ESTERS UNDER FRYING CONDITIONS

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3-monochloro-1,2-propanediol (3-MCPD) esters are widely discussed processing contaminants especially due to the release of a potential carcinogen, free 3-MCPD in vivo from its bound form. While a lot of studies have been concerned with 3-MCPD esters formation pathways, the knowledge of their stability during various processing practices is very limited. In our preliminary experiments, degradation of these contaminants has been observed. More detailed attention was paid to the identification of the major degradation products. For this purpose, ultra-high-performance liquid chromatography (U-HPLC) coupled to high-resolution mass spectrometry (HR–MS) with an Orbitrap mass analyzer was employed. A wide range of degradation products originated during thermal treatment of an isotopically labelled standard of 1,2-dipalmitoyl-3-MCPD-d_5 (1,2-diP-3-MCPD-d_5) as the result of its treatment at 180°C (imitating frying conditions).
The experiments were performed in three main parts:
1) Heating the pure isotopically labelled standard 1,2-diP-3-MCPD-d_5
2) Heating 1,2-diP-3-MCPD-d5 in a neutral model medium (methyl esters of fatty acids) simulating the real oil matrix
3) Heating a real matrix (pure palm oil commonly used for frying)
When heating the pure labelled standard, 1,2-diP-3-MCPD-d_5, a 90% reduction was observed in 45 min. Simultaneous formation and degradation of products originated mainly from the parent compound (mainly due to dechlorination and deacylation) was observed. In the presence of methyl esters, the 90% reduction of 1,2-diP-3-MCPD-d_5 was achieved after 8 hours under the same treatment conditions. When heating palm oil a 90% of reduction of total 3-MCPD esters was observed after 10 hours of heating. The amount of 3-MCPD esters before heating was 3468 µg/kg fat.

Keywords: *3-MCPD esters, degradation products, dechlorination, deacylation*

Acknowledgement: *Financial support from specific university research (MSMT No 20/2015).*

L40
FORMATION OF DIAMINOBENZOQUINONES FROM (ETHYL)VANILLIN IN BAKERY PRODUCTS: SAFE OR UNSAFE?

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The complex chemistry of baked products was deeply studied in the past, particularly regarding the occurrence of the Maillard Reactions, a complex group of reactions allowing the formation of aroma, color and, sometimes, leading the release/accumulation of potentially toxic compounds in foods. Natural or synthetic vanillin (4-hydroxy-3-methoxybenzaldehyde) and the more aromatic and stable synthetic analog ethyl vanillin (4-hydroxy-3-ethoxybenzaldehyde) is one of the largely used flavors in bakery products. Previous data suggested the loss of sweet/flavored aroma intensity of vanillin, supporting the idea that vanillin aroma is affected by food components, especially proteins and amino acids [1]. Considering these preliminary suggestions, we focused our work on the study of the reaction of vanillin (and ethyl vanillin) with amines and amino acids, highlighting the prompt and efficient formation of benzoquinone derivatives, whose color may contribute to the overall browning process beside the Maillard Reactions. In detail, (ethyl)vanillin reacts with secondary amino groups in aerobic (hydro)alcoholic conditions to give good yields of 2,5-diamino-1,4-benzoquinones, characterized by NMR and by comparison with literature data. The mechanism of their formation is discussed, along with the identification of intermediates and byproducts. 2,5-diamino-1,4-benzoquinones were then used as standard compounds for further analyses (formation in model food analysis, as well as toxicity studies). A biscuit was prepared as model food containing conventional quantity of vanillin and spiked with high concentration of suitable amine components as the reactive counterpart. Following bakin g in conventional oven, the compounds were identified in biscuits using HPLC-MS chromatography, confirming their formation in a real food system. Moreover, the toxicity of the compounds obtained from synthesis was evaluated considering different cell lines (LAMA-84 and HT-29) and Comet Assay, for the assessment of the cyto- and genotoxicity. Preliminary data suggest no cytotoxicity of the compounds in colon carcinoma cells up to 20µM. Concluding, in this study we have identified and toxicologically characterized 2,5-diamino-1,4-benzoquinones arising from the reaction of vanillin with secondary amines. Demonstration of their formation in a food model system provided new insights on an alternative chemical pathway for the loss of vanillin and the corresponding decrease of flavoring. Future efforts will be directed to elucidate the whole reaction mechanism and by-products, to explore the reactivity of (ethyl)vanillin towards amines/amino acids either endogenous in food or generated during Maillard processes and, finally, to define the toxicological profiles of these compounds.

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Keywords: *vanillin, processing contaminants, diaminobenzoquinones, bakery products*

L41
AN EFFECTIVE ANALYTICAL STRATEGY TO COMBAT FOOD FRAUD – CHALLENGES FACED BY THE FOOD ANALYSIS LABORATORY AND THE SOLUTIONS PROVIDED

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Increasing globalisation has been identified as one of the main drivers of emerging risks linked to food safety and public health. Unintentional contamination of food products – presence of pesticide residues, mycotoxins, heavy metals – is adequately covered, at least in the developed countries, by regulations providing guidance on maximum acceptable levels for contaminant residues and associated reference methods for their analysis. Intentional contamination, on the other hand, is unpredictable and often goes unnoticed. Under the latest GFSI guidelines to mitigate the risk of food fraud, food operators should put in place a documented procedure clearly indicating when, where and how the integrity of food products entering or leaving the factory is verified. Answers to the questions "when" and "where" can be found from a vulnerability assessment of the supply chain. The "how" component relates to the testing strategy in place which is where the analytical laboratory has a major role to play. There are a number of challenges facing the laboratory when it comes to choosing an appropriate analysis to detect fraud. A request such as "Please check whether this sample is authentic" is not very helpful! A simple commodity like fruit juice, for example, may be adulterated by adding extenders like water, sugar, and cheaper fruit or its label may misrepresent claims of provenance or natural vitamin content. Judging authenticity requires substantial reference data from authentic juices; an added challenge given the natural variability of these products. Using an internal reference, as illustrated by the ^{18}O stable isotope method to detect water addition can often get around the need for a huge data base. Targeting all potential frauds, therefore, requires a battery of different tests, inevitably leading to increasing analytical costs and longer sample turnaround times. The latest non-targeted approach such as the routine screening tools using proton NMR for fruit juice, wine and honey offer an interesting solution. Unfortunately these non-targeted techniques are not formally recognised as the usual criteria for defining method performance are not applicable. Hopefully a solution is on the horizon. However the greatest challenge of all for an analytical laboratory is keeping up with the increasingly devious methods of adulteration, requiring even greater sophisticated analyses. The case of adulterated lemon juice in the market in 2012 illustrates this point well. Simple analysis of isocitric acid levels used to be sufficient to detect the addition of synthetic citric acid. Now multi-isotope fingerprinting, correlating carbon and deuterium isotope ratios, are required to be sure that the product is authentic.
This need to constantly develop, modify and improve analytical techniques in the fight against food fraud will keep analytical chemists in employment for many years to come, and provide a wealth of topics for future RAFA conferences!¶

Keywords: *authenticity, food fraud, stable isotope analysis, non-targeted techniques*

L42
TASTE FROM MOTHER NATURE AND CULINARY ART – ANALYTICAL DECODING BY MEANS OF THE SENSOMICS APPROACH

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The development of healthier food products, for example, reduced in fat, sugar, or salt, respectively, are well-known to induce non-acceptable flavor defects in the products and has, thus, created unexpected flavor challenges for the food industry. In response to the consumers' demand for healthy but tasty foods, novel ingredient discovery is essential to overcome such flavor challenges associated with the production of, in particular, sugar, salt or fat-reduced products. Varying widely across the world, reflecting unique environmental, economic, and cultural traditions, various drying, fermentation, cooking and roasting procedures have been empirically developed during the last millenniums and, since then, the alluring flavor of the dishes prepared do attract consumers on a global scale. In particular, the food manufacturing techniques leading to the most premium tastes promise to contain essential taste compounds and/or taste modulators generated from sensory inactive precursors upon processing of the raw materials. This evolutionary refinement of food manufacturing procedures is, therefore, expected to open an interesting avenue towards the discovery of natural taste systems and taste modulating compounds, which might be applied as natural solutions to overcome flavor challenges associated with the production of, in particular, sugar, salt or fat-reduced products. The presentation will highlight analytical strategies to identify key taste compounds and taste modulators in processed food by means of a SENSOMICS approach.

Keywords: *processed food, taste compounds, taste modulators, SENSOMICS*

L43*
MULTIDIMENSIONAL CHROMATOGRAPHIC TECHNIQUES (LC–GC–GC–GC–PREP) FOR THE COLLECTION OF PURE VOLATILE COMPONENTS FROM COMPLEX SAMPLES

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The present research describes the development of a new versatile multidimensional gas chromatographic preparative system (MDGC–prep), coupled to an LC pre-separation step operated under normal phase conditions, depending on the complexity of the sample analysed. The system was demonstrated to be capable to collect volatile molecules in a wide range of concentrations, in a short time period, while maintaining a high degree of purity for the selected components. Furthermore, the LC dimension allows the injection of higher sample amounts with respect to a conventional split/splitless injector as well as the transfer of simplified samples to the MDGC–prep system. With respect to the configurations already described in recent papers, the current configuration was developed by adding two electronically controlled switching valves both in the first and second dimension. The valves allow the collection of pure components directly in each of these dimensions without any hardware modification reducing the total collection time for the components already resolved in the first or second wide bore column. Furthermore, in such a configuration, the generation of co-elutions coming from the multi-cutting of many components is also avoided. The new system was applied to the isolation of multiple components from different complex samples namely carrot seed oil, and Vetiver and Patchouli essential oils.

Keywords: *gas chromatography, essential oils, multidimensional*

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L44*
PROTON-TRANSFER-REACTION MASS SPECTROMETRY FOR THE STUDY OF THE PRODUCTION OF VOLATILE COMPOUNDS AND THE EFFECT OF FLOUR, YEAST AND THEIR INTERACTION DURING THE BREAD-MAKING PROCESS

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Bread is one of the most consumed products all over the world, which gives it an important socio-economic status in human nutrition and justifies the continuous research and development activities on how to improve its sensory, chemical and industrial characteristics [1]. Volatile organic compounds (VOCs) play a key role in this regard because they take shape during the leavening process and are enhanced upon baking. They help in increasing the volume and producing the shape and the crumb texture, as well as defining the taste of the final product [2]. In this project, Proton Transfer Reaction Mass Spectrometry (PTR-MS), coupled to a time-of-flight mass analyzer (ToF) was applied [3], for the first time, in order to assess the release of VOCs during bread-making and the production of VOCs by different bakery starters. The system included a multifunctional autosampler which permitted the follow-up of the leavening process on a small scale with a typical throughput of 500 distinct data points in sixteen hours. This set-up allowed for a fast, automated and real-time monitoring of the leavening process of bread. The overall course of the reaction was reproducible and enabled us to track the evolution of the production or depletion of a large number of VOCs as well as to discriminate between the different types of yeast preparations. This technique also allowed to tentatively identify major VOCs related to yeast metabolic activity or arising upon baking and more importantly, to point out differences in terms of volatile producti on and evolution kinetics either with time, between yeasts, and even before and after baking [1]. Furthermore, PTR–ToF-MS was successfully applied to analyze the effects of Saccharomyces cerevisiae strains as well as the type of wheat flour used in the bread-making process on VOCs production. The results showed a greater impact of yeast strains over the expected flour influence. This observation was confirmed when the leavened dough samples were baked and the volatile profiles determined. However, the peak-by-peak monitoring followed by a tailored developed statistical approach revealed not only the effect of changing ingredients, but also different kinds of yeast/flour interaction, shedding a new light on the selection of ingredients for each bread recipe depending on the desired volatile profile of the baked product and on the potential of PTR-MS in being a fast high-throughput tool able to analyze protechnological microbes/matrix interaction during food fermentations.

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Keywords: *bread-making, VOCs, PTR-MS, aroma, interaction*

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L45*
SENSORY PROFILE AND IMAGE ANALYSIS OF HOMEMADE BREAD WITH ADDITION OF RECOVERED FOOD BY-PRODUCTS

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According to Food and Agriculture Organization of the United Nations (FAO), approximately one-third of the edible parts of food produced for human consumption gets lost or wasted globally. These by-products (BP) are a cost-effective source of valuable bioactive compounds. In order to obtain value-added breads, four types of agroindustry BP were tested: orange peel (OBP), pomegranate peel and interior membranes (PBP), elderberry skin, pulp and seeds (EBP) and β-glucans from spent yeast (BBP). All BP were subjected to a bleaching pre-treatment and then oven-dried, excepting BBP which were extracted from spent yeast through autolysis and lyophilized. All dry BP were grinded and added to wheat flour at different percentages (g/100g of flour). For control bread (C) BP were not added. Breads were prepared according to a bakery recipe and using home-making bread machines (Moulinex, Groupe SEB, France). Bread making program selected included dough preparation (2h 20m) and cooking (20 minutes). The impact of BP incorporation was evaluated on bread sensory profile and crumb structure. A sensory panel composed by 13 members was trained for descriptive analysis according to the guidelines in the ISO 8586 (2012) and validated through Sensory Triangular tests (ISO 4120, 2000). A list of 20 attributes was selected for breads descriptive profile and a score card was developed to evaluate attribute intensities on 8.5-cm unstructured line scales. In evaluation sessions, bread slice (1.5 cm thickness), including the crust and crumb, was presented to assessors in a 3-digit coded glass covered with a glass lid. Control and BP breads replicates were analysed over eight sessions. To study the crumb structure, two slices (1.6 cm thickness) from five breads of each type were cut and analysed. Images were captured in the RGB (24 bit) standard format using a flatbed scanner (Canon iR2016i, Netherlands) with a resolution of 300 dpi. Each image was processed and analysed using a code written in Matlab R2013a (MathWorks). A single 500x500 pixel field of view was cropped from the image centre and converted to a 256 level grey scale. Image segmentation was performed with different techniques. Cell morphological parameters were analysed. Sensory profile showed that EBP bread was the one with more attributes differences (p0.05). Therefore, OBP, PBP, EBP and BBP can be used as bread ingredients without compromising the sensory characteristics and crumb structure, contributing to valorisation of agroindustry BP.

Keywords: *food chemistry, food by-products, bread, descriptive analysis, image analysis*

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L46
OUT WITH THE OLD, IN WITH THE NEW: NOVEL APPROACHES IN ALLERGEN DETECTION USING MALDI–TOF–—TOF AND MASS SPECTROMETRY

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Allergens have been on the menu of regulators and consumer groups for several years now. While the jurisdictions differ in some of the regulated allergens, most have the 8 major allergens referred to in Codex Alimentarius (General Standard for the labelling of pre-packaged foods (Codex STAN 1-1985, section 4.2.1.4). Ensuring the correct labelling of food products containing food allergens is essential for those affected by food allergies. And while this mainly documentation based, the ultimate proof of absence of allergens is done by analysis. Here, conventional technologies like ELISA and PCR have shown to be applicable for a number of matrices. Work by numerous experts in the field however has shown that these technologies are not infallible. This is especially evident when it comes to processed matrices containing egg and milk. Most recently, the adulteration of ground cumin with peanut has shocked the food industry as well as regulators. Consequently, spices have come under close scrutiny. Regulators in North America and Europe took a closer look and found a number of incidences where paprika and spice mixes were allegedly contaminated or adulterated with almond. While the economic benefit of such adulteration is questionable, it was discovered that the suspected almond was actually an Indian cherry (*Prunus mahaleb*). Interestingly, like the discovery that egg and milk often go undetected by ELISA and PCR in processed products, this discovery was made using mass spectrometry. In a recent statement by ANSSA, the French Food Safety authority, mass spectrometry was identified as reference method for allergen analysis. And yet, there has not been any collaborative trial of MS methods, and the subgroup of the European standardisation Committee (CEN) working group dealing with MS for food allergens is dormant. In the light of previous and recent finding on the fallibility of conventional tests for allergens, would it be prudent to review all existing conventional methods and establish reference standards for allergens using MS methods, following the scheme: out with the old – in with the new?

Keywords: *allergens, adulteration, mass spectrometry*

L47
SPEEDING-UP SAMPLE HANDLING FOR MULTIPLEX MS DETECTION OF ALLERGENIC INGREDIENTS IN PROCESSED FOODS

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Contamination of food products by allergens represents a matter of concern especially for allergic consumers due to the risk of triggering an immunological reaction upon ingestion of allergen-containing foods [1]. Due to the widespread extent of such pathology, and in order to protect the health of sensitive consumers, specific legislation has been issued in different countries on the proper labeling of a restricted list of food allergens whenever added to food [2]. Besides their intentional incorporation into the commodity, a risk of accidental contamination is likely to exist. In this case allergens are defined hidden as they have not been declared on the product label and might unexpectedly reach the end products through several routes [3]. Different analytical methods have been developed in the last years for monitoring food allergen contamination along the food chain. Recently, mass spectrometry (MS) methods [1], have been considered a promising analytical strategy for f ood allergens detection thanks to the advances made in this technology that enables to overcome several restrictions of antibody-based methods, such as ELISA. Among them the risk of false positives, especially when applied to complex or processed food matrices that might cause epitope modification or masking, and the limitations in multiplexing. In the present investigation, a sensitive LC–MS method tailored to the multiplex detection of several allergenic ingredients in a processed food matrix will be described. Cookie was chosen as complex and processed food model, incurred with egg, milk, soy, hazelnut and peanut allergens. Starting from our previous investigation [4], extraction, purification and enzymatic digestion conditions were duly optimized in order to design a relatively fast and easy sample handling procedure which allowed to considerably reduce the time requested for sample preparation before LC-MS analysis. Features of two different MS instrumental set-up, also exploiting High Resolution Mass Spectrometric detection, to monitor 5 allergenic foods in complex foods will be presented. An automated on line pre-enrichment procedure onto a trap-column followed by chromatographic separation was designed enabling the pre-enrichment and partial purification of the candidate markers with challenging LODs obtained.

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[3] Hattersley, S. et al. Food Chem. Toxic. 67 (2014) 255. 4. Monaci, L. et al. J. Chromat. A 1358 (2014) 136.

Keywords: *linear ion trap-mass spectrometry, allergens, high resolution MS, food processing, multiplex analysis*

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L48*
SPECIFICITIES OF ELISA TEST KITS FOR GLUTEN DETECTION

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The only known effective therapy for celiac disease (CD) patients is a lifelong gluten-free diet to prevent small intestinal villous atrophy. According to legislation by the Codex Alimentarius, gluten-free foods must contain less than 20 mg gluten per kg of the product. The storage proteins of wheat, rye, and barley, called gluten in the field of CD research, are comprised of a complex mixture of hundreds of single proteins. These can be classified according to solubility in water, saline or aqueous ethanol. Prolamins constitute the alcohol-soluble fraction of gluten, whereas glutelins are insoluble. Both fractions harbor CD-active epitopes harmful to CD patients. Enzyme-linked immunosorbent assays (ELISA) are currently recommended for gluten analysis, but different commercial test kits show considerable differences depending on assay format, extraction procedures, reference materials used for calibration, and antibody specificities. Most antibodies recognize specific amino acid sequences from certain prolamin types and the gluten content is calculated by multiplying the quantitated prolamin content by a factor of 2. However, detailed, comparative investigations on the specificities of different antibodies against prolamin and glutelin fractions of wheat, rye, and barley are missing. Therefore, prolamin and glutelin fractions were extracted from wheat, rye, and barley flours and characterized by RP-HPLC-UV. After appropriate dilution, the fractions were analyzed by six commercial ELISA test kits based on the R5, G12, Skerritt, and alpha20 monoclonal antibodies as well as two different polyclonal antibodies. The protein contents determined by RP-HPLC were used as a basis to compare the results from different test kits. All test kits gave fairly consistent results for wheat prolamins, but huge differences in antibody specificities were observed for rye and barley prolamins, as well as wheat, rye, and barley glutelins. While the gluten content of some fractions was overestimated by a factor of 8, the gluten content of other fractions, especially glutelins, was underestimated by a factor of 10 or more. These findings highlight the need for a comprehensive immunological or non-immunological multi-method capable of detecting both prolamin and glutelin fractions to guarantee the safety of gluten-free products for CD patients.

Keywords: *celiac disease, ELISA, gluten analysis, gluten-free products*

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L49*
AN LC–MS BASED MULTI-METHOD FOR THE DETECTION OF FOOD ALLERGEN TRACE CONTAMINATIONS IN PROCESSED FOODS UTILISING THE LATEST ADVANCES IN HIGH RESOLUTION (HRMS) AS WELL AS MRM^3-BASED TRIPLE-QUADRUPOLE MASS SPECTROMETRY

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Up to 17% of the European population are estimated to suffer from food allergy, an IgE-mediated overreaction against specific food proteins that may have life-threatening consequences. As reliable and cost-effective analytical methods are needed to ensure safe food production for allergic consumers mass spectrometry is progressively gaining importance for the development of novel approaches to the detection of allergen traces in food. We here present to the best of our knowledge the first comprehensive LC–HRMS multi-allergen-screening method for six different allergic nuts in processed foods. In addition, we demonstrate the potential to further lower LODs by the implementation of the MRM^3-technique provided by the newest generation of triple quadrupole mass spectrometers. Using bottom-up proteomics 44 marker peptides were identified for the detection of almond, cashew, hazelnut, peanut, pistachio and walnut. All marker peptides were demonstrated to be specific for the respective nut allergen via database search (BLAST, UniProtKB-Plants) and experimental analysis of other nut species and food matrices with relevant contamination potential. Using this set of marker peptides identified in our study, we were able to discriminate some food allergens down to the level of protein isoforms. Based on these findings an analytical LC–MS method for contaminations of nuts in processed foods was developed. Using high resolution MS with an LTQ Orbitrap XL the analysis of spiked ice cream samples and statistical validation according to DIN gave limits of detection (LOD) below 12 mg/kg for at least three marker peptides from almond, cashew, hazelnut, peanut and pistachio and below 36 mg/kg for walnut respectively. In order to make LC–MS based allergen detection more widely employable a MRM-based method for triple-quadrupole mass spectrometers, which today are widespread in laboratories, was set up. In this regard the latest focus of our work is the implementation of the MRM^3 feature provided by the newest generation of AB Sciex devices, which allows us to further lower LODs. Our results demonstrate that MS based analysis of allergens can compete with the so far more common methods ELISA and PCR in terms of analytical sensitivity. In contrast to ELISA assays our MS method also provides exact information about the detected protein sequences and allows for multiplex allergen analysis. By using the allergenic proteins as analytical targets it furthermore avoids the risk of false negative results that indirect detection methods like PCR face due to a potential discrimination of DNA and proteins.

Keywords: *allergens, tree nuts, high resolution mass spectrometry, triple quadrupole mass spectrometry, MRM³*

L50
EXPOSURE ASSESSMENT TO MULTIPLE CHEMICALS AND FUTURE MIXTURE TESTING

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Every day, we are exposed to multiple chemicals by several routes of exposure: diet, inhalation and dermal contact. These chemicals may exert toxic effects and therefore risk assessment by evaluation of exposure and toxicity is necessary to monitor and control possible adverse effects on human health. Until recently, risk assessment is mostly performed separately for each chemical, considering only a single route of exposure. However, this simplified risk assessment does not take into account the effect that chemicals may have on each other and their effect on the same target organ. Therefore, there is a need to address combined exposure to mixtures of chemicals as set out in EU Regulation. According to EU Regulation, EFSA is responsible to establish the methodology for combined exposure to multiple chemicals. Recently, EFSA has published the outline of the methodology on the EFSA website, including four EFSA opinions on how based on their toxicological profile and EFSA guidance on how to perform the exposure assessment (1). Within the ACROPOLIS project a model was developed to assess the dietary cumulative exposure to a group of seven triazoles following the EFSA guidance and the results are published in a special issue of Food Chemical Toxicology (2). EFSA started grouping pesticides into cumulative assessment groups based on their toxicological profile for all pesticides affecting the nervous system and all pesticides affecting the thyroid gland. EFSA noticed several gaps in the EU monitoring because some pesticides are difficult to analyze in and are therefore not included in the routine monitoring programs. Filling the identified data gaps might pose a challenge for analytical method development. EFSA also noticed that relevant information on the toxicity of the chemicals is not always available. Consequently, EFSA applied a precautionary principle in their grouping and it was noticed that refinements are possible when data on toxicity becomes available. To this end, the new European funded project EuroMix will apply several *in-silico* and *in-vitro* tools to mixtures of chemicals that can affect the liver, the endocrine system, and the development stage. A number of test systems, including omics technology and classic toxicological testing, will be explored. The most promising tests for mixtures will be verified against results from animal studies, which will be performed according to well-accepted OECD test guidance for single chemicals.

[1] Info Session on Applications - Pesticides – Technical meeting on Cumulative Risk Assessment. Parma 11-02-2014 http://www.efsa.europa.eu/en/events/event/140211.htm
[2] Scientific publications ACROPOLIS project: Food and Chemical Toxicology. Volume 79, Pages 1-80 (May 2015) Toxicity testing and model development for estimating cumulative and aggregate exposure to pesticide residues in Europe Edited by Susan M. Barlow and Polly E. Boon. http://www.sciencedirect.com/science/journal/02786915/79

Keywords: *exposure, multiple residues, mixture testing*

L51
(I) EFSA'S RISK ASSESSMENT ON ACRYLAMIDE IN FOOD

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Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on acrylamide (AA) in food. AA is used as an industrial chemical, but can also be formed when certain foods are prepared at temperatures above 120 °C and low moisture, especially in foods containing asparagine and reducing sugars. The CONTAM Panel evaluated a total of 43 419 analytical results from food commodities collected and analysed since 2010 and reported by 24 European countries and six food associations. AA was found at the highest levels in solid coffee substitutes and coffee, and in potato fried products. Mean and 95th percentile dietary AA exposures across surveys and age groups were estimated at 0.4 to 1.9 µg/kg body weight (b.w.) per day and 0.6 to 3.4 µg/kg b.w. per day, respectively. The main contributor to total dietary exposure was generally the category 'Potato fried products (except potato crisps and snacks)'. Preference in home-cooking can have a substantial impact on the dietary exposure, e.g. depending on the conditions of potato frying, the AA dietary exposure could be increased up to 80%. Upon oral intake, AA is absorbed from the gastrointestinal tract and distributed to all organs. AA is extensively metabolised, mostly by conjugation with glutathione but also by epoxidation to glycidamide (GA). Formation of GA is considered to represent the route underlying the genotoxicity and carcinogenicity of AA. Neurotoxicity, adverse effects on male reproduction, developmental toxicity and carcinogenicity were identified as possible critical endpoints for AA toxicity from experimental animal studies. The data from human studies were not adequate for dose-response assessment. Therefore, the CONTAM Panel considered the data from studies on experimental animals to establish the reference points. The CONTAM Panel performed benchmark dose (BMD) analyses on data for neurotoxicity and on the tumour incidences induced by AA in experimental animals. The CONTAM Panel selected the value of 0.43 mg/kg b.w. per day derived as the lowest BMDL10 for peripheral neuropathy in rats as the reference point for non-neoplastic effects. For neoplastic effects, the CONTAM Panel selected as a reference point the value of 0.17 mg/kg b.w. per day derived as the lowest BMDL10 from data on incidences of Harderian gland adenomas and adenocarcinomas in mice. The available data indicates that AA is of concern with respect to genotoxicity. Therefore, the CONTAM Panel considered it inappropriate to establish a tolerable daily intake and applied a margin of exposure (MOE) approach for the risk characterisation. The Panel concluded that the current levels of dietary exposure to AA are not of concern with respect to non-neoplastic effects. However, although the epidemiological associations have not demonstrated AA to be a human carcinogen, the MOEs indicate a concern for neoplastic effects based on animal evidence.

Keywords: *acrylamide, glycidamide, risk assessment, food, exposure*

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(II) NON-ALLOWED PHARMACOLOGICALLY ACTIVE SUBSTANCES: EFSA'S WORK ON RPA, CHLORAMPHENICOL AND NITROFURANS

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Veterinary medicinal products (VMPs) may only be placed on the market if the residues in animal products do not pose any harm to the consumer. Pharmacologically active substances fulfilling this condition are classified as 'Allowed substances'. Regulation (EC) No 470/20094 stipulates that for non-allowed pharmacologically active substances a reference point for action (RPA) may be established. When residues of such non-allowed substances are detected at or above the RPA, the food is considered not to comply with Community legislation, and should be removed from the market. The European Food Safety Authority (EFSA) was asked by the European Commission (EC) to develop a guidance document on methodological principles and scientific methods to be taken into account when establishing RPAs for non-allowed pharmacologically active substances in food of animal origin. The Panel on Contaminants in the Food Chain (CONTAM Panel) developed a simple and pragmatic approach which takes into account both analytical and toxicological considerations. In addition, circumstances were identified under which the EC might consider it appropriate to consult EFSA for a substance-specific risk assessment; including substances causing blood dyscrasias (such as chloramphenicol) or that are high potency carcinogens (such as nitrofurans). As chloramphenicol and nitrofurans are excluded from the RPA guidance document and taking into account the presence from sources other than use of VMPs, the EC asked EFSA for scientific opinions on the risks related to the presence of chloramphenicol and nitrofurans. Chloramphenicol is implicated in the generation of aplastic anaemia in humans and causes reproductive/hepatotoxic effects in animals. The CONTAM Panel concluded that it is unlikely that exposure to food contaminated with chloramphenicol at or below the RPA of 0.3 µg/kg is a health concern for aplastic anaemia or reproductive/hepatotoxic effects. Chloramphenicol exhibits genotoxicity but, owing to the lack of data, the risk of carcinogenicity cannot be assessed. The CONTAM Panel concluded that, when applied to feed, the current RPA is also sufficiently protective for animal health and for public health, arising from residues in animal derived products. Nitrofurans are rapidly metabolised, occurring in animal tissues as protein-bound metabolites. Nitrofurans and their marker metabolites, generally, are genotoxic and carcinogenic and, also, have non-neoplastic effects in animals. Based on the calculated MOEs, the CONTAM Panel concluded that it is unlikely that exposure to food contaminated with nitrofuran marker metabolites at or below 1.0 µg/kg is a health concern.

Keywords: *non-allowed pharmacologically active substances, reference point for action, nitrofurans, chloramphenicol, MOE*

Acknowledgement: *EFSA wishes to thank the members of the EFSA SWG on non-allowed pharmacologically active substances in food and feed and their reference points for action and the members of the former Panel on Contaminants in the Food Chain (CONTAM Panel 2012-2015 mandate, http://www.efsa.europa.eu/en/contammembers/contampreviousmembers.htm). EFSA and the CONTAM Panel acknowledge all European competent institutions that provided occurrence data on chloramphenicol or nitrofurans in food and/or feed, and supported the data collection for the Comprehensive European Food Consumption Database, as well as the stakeholders that provided toxicity studies, usage levels of carrageenan (E 407), or information on semicarbazide in seaweeds.*

L52*
CRITICAL ASSESSMENT OF MOTHERS´/ NEWBORNS´ EXPOSURE PATHWAYS TO CARCINOGENIC PAHS THROUGH ANALYSIS OF THEIR METABOLITES

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Chronic exposure to certain polycyclic aromatic hydrocarbons (PAHs) is associated with a variety of adverse health effects (carcinogenity, mutagenicity, teratogenicity). In the exposed organisms these compounds are rapidly hydroxylated in the liver and then undergo conjugation, typically to the glucuronides and sulfates, which are excreted in urine or feaces. The aim of this contribution is to summarize the main results from the assessment of body burden of Czech population to PAHs via analysis of their monohydroxylated metabolites (OH-PAHs) in urine. For this purpose the novel analytical approach for the determination of a wide range OH-PAHs including the metabolite of carcinogenic benzo[a]pyrene has been successfully developed and validated on SRM 3673 (non-smoker urine). After enzymatic hydrolysis of conjugates, the OH-PAHs from urine hydrolyzate are isolated with ethylacetate and obtained extract is further purified with d-SPE using the sorbent Z-Sep. Th e final analysis is realized by liquid chromatography (U-HPLC) interfaced with tandem mass spectrometry (MS/MS). This study is a part of the project „Impact of air pollution to genome of newborns (No. 13-13458S)“. More than 600 urine samples were collected from Czech women and their newborns living in two regions (České Budějovice and Karviná) differing in atmospheric contamination by PAHs during two periods (i) summer/autumn 2013 and (ii) winter 2013/2014. Since humans are exposed to PAHs by various ways, both dietary exposure and atmospheric pollution has been taken into consideration. Based on the concentrations of PAHs assessed in air (sampled by HiVol samplers of PM2.5), one week diet of pregnant women and human breast milk, exposure data for mothers and newborns will be introduced in presentation. From the 11 targeted analytes, only naphthalen-2-ol was quantified in all urine samples. Other analyzed compounds, represented by metabolites of naphtalen, fluoren, phenanthren and pyren were found in more than 70 % of samples. Chrysene-6-ol and benzo[a]pyren-3-ol were not detect ed in any of the tested urine extracts. In general, the concentrations of OH-PAHs in children urine (median 5.0 µg/g of creatinine) were 1.6× lower compared to their mothers (median 8.0 µg/g of creatinine).

Keywords: *PAHs, OH-PAHs, exposure assessment, human urine*

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L53
STATEGIES FOR MITIGATION OF CONTAMINANTS IN FOOD

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Contamination of food generally has a negative impact on the quality and may imply a risk to human health. Mitigation measures can minimise the contaminant exposure by changes in the primary production, food processing or dietary recommendations. The best strategy depends on the specific problem. Here are three examples: There is a worldwide concern about dietary inorganic arsenic (iAs) exposure since long-term intake has been associated with a range of health problems, including skin lesions, cardiovascular diseases and some forms of cancer (EFSA 2014, FAO/WHO 2011). Food and drinking water are the main sources of exposure for the general population in Europe. The main source with the highest iAs concentration is rice. Changes in agricultural practice (environment, rice variety and color, and grain size), processing (polishing, boiling practice) and dietary recommendations (avoid rice crackers) can reduce the dietary exposure rice products (Sharma et al 2014). In fish fillet production the byproducts are at present turned into ensilage and sold as low priced animal feed. To increase the value of these byproducts high quality omega-3 fish oils and protein products intended for human consumption may be produced. Of cause it should comply with the existing EU maximum levels for heavy metals and dioxins. The aquaculture practice (feed, size, age, fat content) and by-product fraction (intestine or head, tail and bone) influence the contamination level in the raw material. For removal of dioxins deodorization of fish oil at high temperature is recommended. Substituting marine oil in the feed with plant oil will not only decrease dioxins but also the omega-3 level significantly. Although pesticide residues seldom exceed the maximum residue limits (European Commission 2002) consumer awareness is high. Home processing can in some cases reduce the pesticide residues e.g. in apples by washing, boiling, peeling and juicing (Rasmussen et al 2002). The dietary risk assessment can be refined by taking into account changes in contaminant level during processing because of more accurate estimates of the actual consumer exposures. However the agricultural practice, pre-harvest interval from last application of pesticide to harvest, pesticide properties and weather will not only influence the residue level but also alter the effect of home processing practices.

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Keywords: *inorganic arsenic in rice, metals and dioxins in fish, pesticides in apples, mitigation, food processing*

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L54
ALTERNATIVES FOR BISPHENOL A: SHOULD WE BE CONCERNED?

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Bisphenol A (BPA) is a well know high production volume chemical (HPVC) used for the production of polycarbonate plastics, protective linings in e.g. cans and other products (EFSA, 2015). The high volume usage of BPA led to the contamination of food, either directly or indirectly by leaching out of food contact materials, e.g. can linings. The safety of BPA is intensively debated by scientists, non-governmental organisations, industry representatives and policy makers. While the debate (EFSA) is on-going, some countries already put a ban on BPA and the acceptance of BPA by the public seems declining given the marketing of so-called 'BPA-free' products such as drinking bottles. In the meantime, many chemicals with a BPA-like structure ('BPA-analogues') are produced, which may serve as alternatives for BPA. However, although the toxicology of these analogues is also not fully established, several exhibit similar in vitro endocrine activities as BPA (EPA, 2014 and Wang, 2014). It is therefore essential to evaluate (i) the endocrine potencies of these compounds and (ii) their occurrence. Here we present the in vitro determined estrogenic and anti-androgenic potencies of over 20 BPA-analogues and a sensitive and selective chemical multimethod to determine their presence in complex mixtures, e.g. extracts prepared from plastic bottles. The results of the yeast estrogenic and androgenic bioassays showed that several BPA analogues, like BPB, BPC and BPE, showed a higher estrogenic and/or anti-androgenic potency than BPA itself. In order to analyse the possible presence of these analogues in samples like fish and 'BPA-free' products a LC–MS/MS method was optimised for a wide range of BPA analogues using a Waters Acquity UPLC system coupled to a Waters Xevo TQS MS system. Both LC and MS/MS parameters were optimised. Migration experiments are planned for 'BPA-free' bottles, in order to see if analogues with a possible toxicological concern are used as BPA alternatives. Furthermore, fish samples collected over the course of several years are analysed to investigate if BPA levels are declining while levels of alternatives are increasing.

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Keywords: *BPA, LC-MS/MS, hormone bioassay, endocrine active*

L55
INTERACTIVE SEMINAR – BE ACTIVE AND LEARN FROM EACH OTHER: SAMPLE-PREP, SEPARATION TECHNIQUES AND MASS SPECTROMETRIC DETECTION IN FOOD QUALITY AND SAFETY: STEP BY STEP STRATEGIES TOWARDS FAST DEVELOPMENT OF SMART ANALYTICAL METHODS

Moderators:

Kateřina Maštovská[1], Hans Mol[2], Milena Zachariášová[3]

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This educative seminar is intended for young scientists but all other RAFA attendees are also welcome! It will provide interactive demonstration of general approaches to fast development and troubleshooting of analytical methods for food quality and safety control. The moderators will introduce several case studies with various conceivable scenarios for each step in the method development (including both sample preparation and instrumental analysis) and/or for each troubleshooting problem. Each time, the attendees will identify the most suitable solution using an anonymous electronic voting system, followed by an interactive discussion about each presented option. In the end of the seminar, groups of participants will compete against each other by proposing optimal solutions for particular analytical problems. The best ideas will be awarded by special prizes!

We encourage you to attend this informal and interactive seminar, which was very well received at RAFA in 2011 and 2013. Come to join the discussion, outline your vision, learn something new and have some fun!

All attendees on the board through your voting device!

Please note the capacity of the seminar is limited.

L56
HOW DOES CLIMATE CHANGE IMPACT ON THE OCCURRENCE AND THE DETERMINATION OF NATURAL TOXINS

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In the meanwhile, it is indisputable that climate change takes place. The increasing levels of greenhouse gas emissions have significantly contributed to a rise of the global mean temperature by 0.89°C in the last century. Food safety and security could be profoundly compromised under changing climate scenarios. Under extreme weather conditions such as heat, drought and heavy rainfalls, fungal diseases and algal blooms have become increasingly unpredictable. Despite huge research investments, prevention and control of these toxic secondary metabolites remains difficult and the agriculture and food industries continue to be vulnerable to problems of contamination and especially in view of issues related to climate change: In 2013 Romania, Serbia and Croatia, reported aflatoxin M1 contamination of milk. Severe droughts in Serbia in 2012 resulted in 70% of the maize crop being contaminated with aflatoxins. Use of this maize to feed dairy cattle led to the high levels of aflatoxin M1 in milk, up to twice the EU legal limit. On the other hand, the catastrophic floods and the rainy summer in 2014 resulted in low levels of aflatoxin B1 with high levels of DON. Obviously, extreme weather conditions as a result of climate change is increasingly affecting the mycotoxin map in Europe and also world-wide. To the unpredictability of the range of mycotoxins occurring in food crops, there is an increasing need for LC–MS based multi-analyte testing methods to check for a wide spectrum of possible secondary metabolites in the food and feed chain. In addition, powerful metabolomics approaches are required to reveal novel findings on the plant-fungi interactions as a result of climate change and resistance breeding. Still, there is also a demand for improved rapid on-site methods and reference materials for a wide range of toxic secondary metabolites including so-called “emerging toxins” and masked mycotoxins. Utilizing the power of multi-toxin screening of food and feed commodities this paper summarizes trends and amazing new findings which can be traced back to climate change and recent efforts in resistance breeding. The paper will also report on the increasing threat through paralytic shellfish toxins as a result of global warming.

Keywords: *climat change, natural toxins, multi-toxin screening, food and feed commodities, LC–MS and rapid on-site analytical methods*

L57
AN INTEGRATED STRATEGY FOR MARINE TOXINS OF CELL BASED BIOASSAYS AND ANALYTICAL TOOLS TO ENSURE SAFE SEAFOOD

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Marine toxins are toxins produced by algae that can accumulate in seafood. Consumption of contaminated products may lead to intoxication such as memory loss, paralysis, diarrhoea or in severe cases death. In order to protect consumers, methods have been developed and put into practice. Within Europe several alternatives for the widely applied mouse bioassay (MBA) are implemented within legislation, i.e. LC–UV, LC–FLD and LC–MS/MS for respectively amnesic, paralytic and diarrheic shellfish poisoning toxins. Current trends involve rapid LC–MS/MS for all the various toxin classes or targeted screening with LC-hrMS for a wide variety of regulated and unregulated toxins. Despite the current alternatives and developments, the unethical and unreliable test with mice is still being used, as it is capable to detect possible unknown toxins or new risks for consumers. Animal-free in vitro cell based effect assays offer the same opportunity as the mouse bioassay, i.e. to detect unknown toxins and new risks. A neuroblastoma cell assay was optimized at our laboratory for the detection of various toxins in seafood samples. This easy and relatively cheap in vitro cell assay is used as a first screening to differentiate between blank and suspect samples. Only the suspect samples are further investigated with analytical chemical tools (LC–MS/MS). For the regulated and some non-regulated toxins such targeted LC–MS/MS methods have been established, and when necessary can be applied in routine monitoring programs themselves (e.g. if countries miss laboratories with cell culture facilities). However, the combination of effect screening with LC–MS/MS confirmat ion is very powerful. If unexplained results are obtained, i.e. suspect samples in the cell assay that cannot be confirmed by LC–MS/MS, a second stage of cell based assays is applied which are more directed to the specific mode of action. For diarrheic marine toxins, these techniques are based on gene expression profiles in human intestinal Caco-2 cells, and a multielectrode array with rat neural cells was set-up for neurotoxic compounds. If suspect samples also show a response in these specific cell assays, and thus suggest the presence of an unknown toxin, analytical tools are used to identify the toxic compound, e.g. LC–HRMS in combination with library searching and/or statistical tools in combination with structure elucidation tools. This strategy has been applied to over 100 mainly contaminated samples. The majority of the suspect samples could be confirmed by LC-MS/MS and contained clearly elevated levels of marine toxins, and till now only one false negative was observed. The assay also flagged some as suspect that could not be explained by the LC–MS/MS methods. These samples are currently under investigation with the multielectrode array. Results indicate that the complete proposed strategy, effect based assays combined with novel analytical tools, will be able to bypass the MBA.

Keywords: *marine toxins, N2a-assay, LC–MS*

L58*
UNEXPECTED DEOXYNIVALENOL QUANTIFICATION ERROR: BE NEVER SURE WITH YOUR UNIT RESOLUTION DATA

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Deoxynivalenol (DON), an epoxy-sesquiterpenoid from the group of type B trichothecenes, belongs to the most frequently occurring mycotoxins in cereals. Both screening and (semi-)quantitative methods based on a specific interaction between antigen and antibody, and also methods utilizing sophisticated instruments based on (ultra/ultra-high performance) liquid chromatography separation and suitable mass spectrometric detection, are usually used for its analysis. Slight disadvantage of the immunoassays can be their cross-reactivity with structurally similar sample components, which may lead to overestimated results. On the other hand, sensitive and selective unit/high resolution mass spectrometry usually provides more reliable results. Nevertheless, some times, also the LC–MS results can be influenced by certain type of matrix effects, possibly leading to the quantification bias.
The presented case study describes the troubleshooting we have to solve after the problems with results reported within the proficiency inter-laboratory testing. The cause of the problem was the unexpected interferences at the masses 310 and 279 *m/z* (referring to the ^{13}C isotopically labeled deoxynivalenol fragment ions) originating from the sample matrix. It should be noted, that the interfering matrix ions were present at the quantitative, as well as at both confirmatory ion transitions detected by the triple quadrupole unit resolution mass spectrometry. The artificially increased signal of isotopically labelled DON resulted in significant overestimation of DON results. The problem was satisfactorily elucidated by enabling of high resolution tandem mass spectrometry (Q-orbitrap), which was able to resolve these very close masses of ^{13}C-analyte and interferences, thus eliminate the interference contribution by applying a very narrow mass extraction window (5 ppm) for chromatograms mining. This practical troubleshooting example just underlines the necessity of confirmation of results by combination of different mass spectrometric approaches available. In this particular case, the unique selectivity of high resolution mass spectrometry helped to avoid the quantification error.

Keywords: *deoxynivalenol, quantification, U-HPLC–MS/MS, U-HPLC–HRMS/MS, Q-orbitrap*

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L59
AN INTEGRATED QUAN AND QUAL STRATEGY BASED ON TRIPLETOF HRMS FOR HOLISTIC DETERMINATION OF ERGOT ALKALOIDS IN CEREALS

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Ergot alkaloids are mycotoxins produced by fungi of the Claviceps genus, mainly by Claviceps purpurea. Infections are mostly prevalent in cereals and wild grasses. After the infection is established, the fungus replaces the developing grain or seed with an alkaloid-containing hard black tuber-like wintering structure called sclerotium, also known as ergot. The alkaloid pattern and individual alkaloid contents in sclerotia vary largely, due to differences in the maturity of the sclerotia and to other factors such as the fungal strain, the host plant, the geographical region and the prevailing weather conditions. Consumption of contaminated food and feed commodities leads to various adverse health effects.
An ultra-high performance liquid chromatography (UHPLC) hybrid quadrupole – time of flight (Q-TOF) mass spectrometry (MS) method is described for the simultaneous quantitative determination of common ergot alkaloids and the screening, detection and identification of unexpected (less studied or novel) members of this class of toxic fungal secondary metabolites. The employed analytical strategy involves an untargeted data acquisition (consisting of full scan TOF MS survey and information dependent acquisition (IDA) MS/MS scans) and the processing of data using both targeted and untargeted approaches. Method performance characteristics for the quantitative analysis of 6 common ergot alkaloids i.e. ergometrine, ergosine, ergotamine, ergocornine, ergocristine, ergokryptine and their corresponding epimers in rye were comparable to those previously reported for triple-quadrupole (QqQ) MS/MS. The method limits of quantification (LOQ's) were in the range from 3 to 19 µg/kg, and good linearity was observed for the different ergot alkaloids in the range from LOQ to 1000 µg/kg. Furthermore, the method demonstrated good precision (RSD's at 50 µg/kg not higher than 14.6% and 16.2% for the intra-day and inter-day precision, respectively), and the trueness values at different concentration levels were all between 89 and 115%. The method was applied for the analysis of a set of 17 rye samples and demonstrated the presence of these ergot alkaloids in the range from < LOQ to 2811 µg/kg. Further mining of the same data based on a 'non-targeted peak finding' algorithm and the use of full MS and MS/MS accurate mass data allowed the detection and identification of 19 ergot alkaloids that are commonly not included in most analytical methods using QqQ instruments. Some of these alkaloids are reported for the first time in naturally contaminated samples.

Keywords: *ergot alkaloid identification, targeted and untargeted analysis, TOF-MS–IDA MS/MS, TripleTOF*

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L60*
METABOLIC FATE OF THE FUSARIUM MYCOTOXINS T-2 AND HT-2 IN WHEAT

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The Fusarium mycotoxins T-2 toxin (T2) and HT-2 toxin (HT2) are classified as type-A trichothecenes and are frequent contaminants of cereals including wheat. They can inhibit RNA, DNA and protein synthesis and due to their toxic potential the European Food Safety Authority proposed a tolerable daily intake of 100 ng/kg body weight for the sum of T2 and HT2. Whereas the mammalian metabolism of T2 and HT2 was already studied in several species, information about the metabolic fate of these mycotoxins in plants and the formed metabolites (masked mycotoxins) is limited.
In this study, an untargeted metabolomics approach utilizing stable isotopic labelling and liquid chromatography-high resolution mass spectrometry (LC–HRMS) was performed. Wheat ears of the variety Remus (susceptible to Fusarium head blight (FHB)) were treated with a mixture of isotopically labelled and non-labelled T2, labelled and non-labelled HT2 or with acetonitrile:water (50:50, *v/v*) as control at several time points. Sampling was performed 24 h after the last treatment and the ears were shock-frozen in liquid nitrogen, ground, extracted and analysed by LC–HRMS. The in-house developed MetExtract software was used for identifying HT2 or T2 derived metabolites and annotation was based on the accurate mass measurements, as well as LC–HRMS/MS spectra. In total, 11 HT2 and 12 T2 derived *in planta* biotransformation products were putatively annotated. In addition to previously reported mono- and di-glucosylated forms of HT2, for the first time HT2-malonyl-glucoside and feruloyl-T2, as well as acetylation and de-acetylation products were detected in wheat. In order to monitor the kinetics of formed metabolites, a time course experiment was conducted involving the variety Remus and the FHB resistant cultivar CM-82036 and eight time points ranging from time point zero to full ripening. Already at the first time point (6 h after treatment), the first biotransformation reactions were observed. De-acetylation of T2 to HT2, as well as glucosylation of HT2 occurred rapidly. While most biotransformation products showed a maximum abundance after ripening, 3-acetyl-T2 and feruloyl-T2 abundances showed the highest levels one day after treatment. After ripening, less than 15% of the toxins added to the plants remained unmetabolized. The same metabolites were observed in the FHB resistant wheat line CM-82036 and the FHB susceptible wheat line Remus. Further investigations to compare the role of detoxification efficiency of wheat varieties in FHB resistance are warranted.

Keywords: *masked mycotoxins, liquid chromatography-high resolution mass spectrometry, untargeted metabolomics approach, cereals*

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L61
RELEVANCE OF MODIFIED MYCOTOXINS IN FOOD: STATE OF THE ART AND FUTURE CHALLENGES

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The European Food Safety Authority (EFSA) has recently published a scientific opinion about the occurrence of modified forms of mycotoxins in food and feed. Based on the literature data so far, modified forms of mycotoxins may add substantially to the overall mycotoxin levels, in particular for zearalenone and fumonisins. Therefore, and in consideration of the possible release of the parent compound in the gastrointestinal tract of humans and animals, reliable data about occurrence and toxicological effects are required. Anyway, these topics can be properly addressed only if fit-for-purpose analytical protocols are employed. Besides instrumentation, the development of proper extraction and sample clean up is still an open issue. While the collection of reliable occurrence data strongly needs calibrants and reference materials on the market, the identification of novel compounds and their mechanism of formation calls for -omics approaches. Toxicological studies at molecular level imply, on the other side, a deeper knowledge of metabolic processes, often supported by in vitro and in vivo studies. In this case, sensitivity and accuracy in identification are key factors. Finally, the development of immunochemical methods based on indirect protocols or antibodies cross-reactivity may represent an opportunity for the implementation of simpler methodologies to be used as screening techniques. However, although steps have been taken in this direction, feasible solutions for routine analysis are still to be proposed. Moving to the most recent literature, this communication will cover the current knowledge on masked/modified mycotoxins in food and feed, with particular emphasis on the analytical challenges that have to be faced in the near future.

Keywords: *masked mycotoxins, mass spectrometry, food safety*

L62
LC–MS/MS BASED ANALYSIS OF HUNDREDS OF MYCOTOXINS: HOW DOES IT PERFORM AND WHAT IS IT GOOD FOR?

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LC–MS/MS based analysis of hundreds of mycotoxins: How does it perform and what is it good for? In the recent years, liquid chromatography-tandem mass spectrometry (LC–MS/MS) has been successfully applied for the analysis of multiple pesticides, pharmaceuticals, veterinary drugs and natural toxins. However, there are still concerns about the limited accuracy of this approach, as matrix effects might not be effectively under control. This is especially true in case of methods targeting hundreds of analytes that are based on the analysis of diluted crude extracts as some of the target substances might not be amenable to the chosen clean-up procedure. For this reason, the method that we have developed for hundreds of fungal metabolites during the last few years has been designed to minimize matrix effects rather than to obtain maximum sensitivity. This is obtained by using conventional HPLC in connection with a large flow rate and a relatively low injection volume. The results that have been obtained over the past years in proficiency testing indicate that this dilute and shoot approach may compete in terms of accuracy with conventional methods. Some authors have expressed the concern that analysing fungal metabolites beyond the range of mycotoxins addressed by regulatory limits is of little or no relevance in view of food and feed safety. For this reason another aim of this presentation is to prove the added value of our method as we have experienced both extreme individual cases (e.g. 125 ppm citrinin in a maize sample) as well as relevance of certain analyte/matrix combinations such as sterigmatocystin in cocoa.

Keywords: *mycotoxins, LC–MS/MS based multi-analyte methods, proficiency testing*

L63
IDENTIFICATION AND TOXICOLOGICAL CHARACTERIZATION OF DEOXYNIVALENOL-3-SULFATE, A NEWLY DISCOVERED METABOLITE OF THE MYCOTOXIN DEOXYNIVALENOL IN HUMAN URINE

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The trichothecene mycotoxin deoxynivalenol (DON) is a frequent food contaminant and has been associated with gastroenteritis and primary symptoms such as nausea, diarrhea, and vomiting in humans. DON is extensively metabolized in plants, animals, and humans resulting in metabolites of diverse chemical and toxicological properties. In humans glucuronidation and de-epoxydation, but not sulfation have been described as routes of DON conjugation/detoxification. In fact, no analytical methodology for the detection of the latter in human samples was available so far. Here, we report the development of a highly sensitive LC–MS/MS method for the quantification of DON-sulfates together with other relevant DON metabolites in human urine for advanced bio-monitoring purpose. Authentic DON-sulfate standards were chemically synthesized and their structure was confirmed by NMR. For sample preparation a 'dilute and shoot' approach was chosen. Despite urine dilution convenient limits of detection below 0.6 ng/mL were achieved for DON-3-sulfate and DON-15-sulfate. The developed method was applied to a set of urine samples obtained from pregnant women who were exposed to DON through their normal diet. Many of the samples have been previously shown to contain DON in concentrations up to 275 ng/mL and even higher concentrations of DON-glucuronides. Using the developed method we were able to detect DON-3-sulfate in 29 out of 40 urine samples (72%). The maximum and median concentration of DON-3-sulfate was 56 ng/mL and 1.5 ng/mL, respectively contributing to about 3-4% of the total DON content in the tested samples (i.e. sum of DON, DON-3-glucuronide, DON-15-glucuronide, and DON-3-sulfate). Interestingly, no DON-15-sulfate was detected in any sample. Though the absolute concentrations of DON-3-sulfate were lower than those of DON-glucuronides, the detection of this novel metabolic pathway in humans opens up for new research questions. In fact, the contribution of sulfate metabolites to the overall toxicity of DON remains to be clarified. Therefore, in vitro experiments were performed for a first toxicological characterization of DON-sulfates in human HT-29 colon cells. Several assays were carried out focusing on cellular oxidative stress response (ROS production), and a potential activation of the Nrf2/ARE pathway. The overall results of these preliminary experiments suggest that the formation of DON-3-sulfate might not be considered as a human detoxification mechanism. In view of the first identification of this metabolite in human urine these results demand a more detailed investigation of this newly discovered conjugate of the Fusarium toxin DON in human.

Keywords: *LC–MS/MS, bio-monitoring, phase II metabolism, biomarker of exposure, deoxynivalenol conjugate*

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L64*
ZEARALENONE-14-O-GLUCOSIDE TAKES OFF THE MASK: HYBRID IN SILICO / IN VITRO APPROACH TO INVESTIGATE THE MOLECULAR BASIS OF ITS XENOESTROGENIC POTENTIAL

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Zearalenone (ZEN) is a mycoestrogen produced by Fusarium spp, mainly F. culmorum and F. graminearum infecting small-grain cereals and maize worldwide, which may enter the food and feed chains. Due to major health concerns – mostly referable to the onset of sexual disorders and alteration of development in humans and animals – ZEN occurred has been regulated by EU in unprocessed cereals and products thereof, assuming a TDI of 0.25 µg/kg b.w. Besides ZEN, several plant metabolites have been discovered in the recent years, among them the modified form ZEN-14-O-glucoside (ZEN-14-Glc). These modified forms are still totally unregulated and largely overlooked from a toxicological point of view. These conjugates may indeed co-occur with the parent compound in a relevant ratio. In the lack of a whole toxicological profile, the scientific community is struggling since years about the toxicological relevance of these modified forms. In parallel, relying on the few data available, the conjugation pathway in plant is claimed as a promising route to increase plant resistance and, thus, mitigate the mycotoxin content. In such a scenario, this study states for the first time the ability of ZEN-14-Glc to trigger a xenoestrogenic response comparable to ZEN in cell-based in vitro assays (based on yeast and MCF-7). Surprisingly, in a cell-free binding assay a minimal affinity for the estrogen eceptor alpha observed was observed – the activation of which is crucial for initiating the (xeno)estrogenic response. At first glance, the stark contrast between cell-free binding and activity observed in cells suggested a possible prominent role of cell transports in balancing the intracellular level of ZEN and ZEN-14-Glc and/or a chemotype-specific cellular response in activating the transcriptional machinery. However, even if these mechanisms cannot be excluded at all, in silico analysis revealed the incapability of ZEN-14-Glc per se to act as agonist on the basis of an unequivocal pharmacophoric mismatch with the agonist conformation of the estrogen receptor pocket. Thereversion to ZEN has been hypo thesized accordingly and it has been investigated and further confirmed in MCF-7 cell line. In addition to remark the effectiveness of in silico modeling in understanding the molecular mechanism of action, our finding strongly support the precautionary inclusion of this masked form in risk assessment studies, since it might concur to the total ZEN-dependent (xeno)estrogenic load. It is worth mentioning that our outcomes are in apparent contrast with what previously reported for glucuronidated forms for which the reversion to ZEN was not observed, thus suggesting a chemotype-specific fate for glycosylated compounds.

Keywords: *zearalenone, masked mycotoxins, xenoestrogens, molecular modeling, estrogen receptor*

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L65*
APPLICATION OF ION MOBILITY Q–TOF LC/MS PLATFORM IN MASKED MYCOTOXINS RESEARCH

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Ion mobility spectrometry (IMS) is a technique enabling the separation of ions according to their different mobilities in the gas phase under the influence of an electric field [1, 2]. Its combination with mass spectrometry and liquid chromatography brings an additional dimension of separation (based on the size and shape of the ionized analyte molecules) with a high application potential for both the target and non-target analysis of complex matrices. Our aim was to utilize the advantages of IMS for the characterization of masked mycotoxins, with the main focus on the sugar conjugates of deoxynivalenol (DON). As our recent research shown, not only mono- but also oligosaccharides can be a part of the deoxynivalenol conjugates. They were identified as abundant masked contaminants of beer and are considered to be a potential food safety issue requiring continuous investigation [3, 4]. As the bioavailability of the conjugates can fairly depend on the chemical bond both between the toxin and (oligo) saccharide molecules and also within the oligosaccharide moiety itself, the estimation of isomeric species number and elucidation of their structure is essential to perform an accurate risk assessment. By implementing the platform of uniform field ion mobility spectrometry coupled with ultra-high performance liquid chromatography and high resolution mass spectrometry (Agilent 6560 Ion Mobility Q–TOF LC/MS System) we were able to: *(i)* resolve the structural isomers, which cannot be separated by common chromatographic setup, *(ii)* directly determinate the collision cross-section (CCS) values for the isomeric species differing in the molecule shape (under practical conditions, the CCS value can be used as an identification point for confirmation purposes), and *(iii)* detect and identify even the trace compounds thanks to fairly improved spectral purity and increased signal intensity.

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Keywords: *ion mobility Q-TOF LC/MS, collision cross-section, masked mycotoxins, deoxynivalenol, deoxynivalenol oligoglucosides*

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L66
PYRROLIZIDINE ALKALOIDS IN OUR DAILY FOOD – OUTCOMES OF AN ACROSS-EUROPE SURVEY AND IMPLICATIONS FOR FOOD SAFETY

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1,2-Unsaturated pyrrolizidine alkaloids (PAs) are secondary metabolites produced by a wide variety of plants from the families of Asteraceae (Senecio, Eupatorium), Boraginaceae and Fabaceae (Crotalaria). PAs are regarded as highly undesirable substances in food and feed, due to their genotoxic and carcinogenic properties, and for that reason have been the subject of two EFSA opinions (2007, 2011). Due to the limited availability of suitable occurrence data in food products (only substantial data for honey were available), in 2013 a large survey was initiated and funded by EFSA, to investigate the occurrence of PAs in animal-derived food products including milk, eggs and meat products, and in plant-derived food products including (herbal) teas and food supplements, across different regions in Europe. Over 1100 samples were collected between January 2014 and April 2015 in 6 different European countries and analysed for the presence of PAs. Two analytical LC–MS/MS based methods, in-house validated and performing at the low levels that were required, were used to quantify 35 different PAs in animal-derived samples and 28 different PAs in plant-derived samples. Limits of detection were in the order of 0.007–0.025 µg/L in (herbal) tea infusion, 0.03–0.25 µg/kg in the animal products and 0.3–2.3 µg/kg in herbal supplements. Analysis of the animal-derived products revealed the occasional presence (6%), albeit at low levels and mostly of single PAs, in milk samples. PAs were practically absent in eggs and meat samples. In contrast, a high proportion of teas (91%) contained one or more PAs. The mean concentration for the sum of 28 PAs was 6.13 µg/L tea infusion, with a maximum of 64.0 µg/L. Of the various types of tea infusion, rooibos tea contained approximately twice the PA amount of that of chamomile tea. 20 different PAs contributed for at least 0.5% to the total PA contamination in tea, the most prominent being senecionine, retrorsine and seneciphylline and their N-oxide forms. Food supplements were often contaminated with PAs (60%), but the concentrations were highly variable. As expected, the highest levels were found in herbal food supplements made from plant material of known PA producers. Supplements containing oil-based extracts of PA-producing plants were generally free of PAs. In the food supplements, lycopsamine, intermedine and echimidine were the PAs most frequently found. Based on limited available toxicological data, in 2011 a margin of exposure (MOE) of 1:10,000 was estimated by EFSA for a daily exposure of 7 ng PAs/kg body weight. Although the calculation was based on a worse case approach, it is evident from the collected data that in particular the contamination of teas presents a major issue, as daily consumption of a single cup (150 ml) in 40% of cases already could result in a MOE smaller than 1:10,000. The consequences for food safety and control will be discussed as well as suggestions made for further research.

Keywords: *pyrrolizidine alkaloids, survey, animal-derived, plant-derived, exposure*

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L67
ANALYSIS OF NANOPARTICLES IN FOOD, COSMETICS AND CONSUMER PRODUCTS

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Nanoparticles are currently present in many products of everyday use. Titanium dioxide particles as UV filters in sun screen lotions, nano-sized fractions of approved particulate food additives such as silica (E551) and titania (E171), colloidal silver as preservative in household cleaners and nano-encapsulated anti-oxidants for use in food and cosmetics are just some examples. In the EU, several regulations already address the presence of nanomaterials in food and products within the approval procedure, the safety assessment, and/or by a labelling obligation. These regulations cover i.a. biocidal products, cosmetics, medical devices, (novel) food and food additives as well as food contact materials. For instance, the labelling of nanomaterials on the ingredient list is obligatory for cosmetics (since 2013) and for food (since 2014). Reliable analytical methods for the detection and quantification of nanoparticles in food, cosmetics and products are thus required both for exposure assessment in the framework of risk assessment/management as well as for the enforcement of existing regulations. In recent years a number of methods have been developed that are capable of detecting and quantifying nanoparticles in food and related matrices. The developed approaches include sample preparation aspects, imaging techniques such as electron microscopy, separation methods (e.g. field flow fractionation, hydrodynamic chromatography, centrifugation) and detection/ characterisation techniques (e.g. light scattering, mass spectrometry). The current state of the art will be reviewed in the presentation and highlighted with some examples. Further improvement and consolidation of the analytical capabilities are still priorities. Techniques have to be improved (i.a. with view to particle size detection limits and particle counting according to the EC definition of nanomaterial), further methods for specific analyte/matrix combinations need to be developed and the harmonisation of data quality and thus confidence in results has to be improved, e.g. by provision of suited reference materials, interlaboratory method performance studies, harmonised validation guidelines and standardised methods. Respective efforts are currently ongoing, i.a. in the EU research project NanoDefine. The progress will be presented.

Keywords: *nanoparticles, sample preparation, separation, validation, NanoDefine*

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L68
APPLICATION AND FUTURE PERSPECTIVE OF AUTOMATED ELECTRON MICROSCOPY TO QUANTIFY ENGINEERED NANOPARTICLES IN COMPLEX MATRICES

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Increasing amounts of engineered nanoparticles (ENPs) already found their way into a range of consumer products, such as sunscreen, food products and textiles. Although the use and incorporation of ENPs into consumer products leads to beneficial properties of the respective products, the risks associated with the exposure and ingestion of these novel materials are still only poorly understood. Research on the effects and interaction of ENPs with biological systems is hampered by the lack of analytical technique to detect and quantify ENPs in complex matrices. Most frequently applied bulk technique, such as dynamic light scattering or disc centrifugation are inappropriate for complex matrices (e.g. presence of other particulate materials) and lack chemical information, which makes respective results very challenging to interpret. Recent developments rely on the 'counting' of individual particles and thus the signals are not biased towards any size fraction. Most promising methods include single particle ICP–MS analysis and highly resolved imaging techniques (e.g. electron microscopy) combined with image analysis tools, the latter being the focus of this presentation. Although the electron microscopy is a rather old technique and sub-nanometer resolutions have been achieved decades ago, the lack of automated analysis routines (including both recording and analysis) makes the technique very labor and thus cost intensive. However, recent improvements in electronics and detector design resulted is faster image acquisitions and more robust long term (several hours) operations, which substantially reduce the required manpower. In combination with tailored image analysis routines (e.g. developed within the FP7 project NanoDefine) the cost per analysis can be substantially be reduced. In this presentation we will demonstrate the possibilities of automated electron microscopy operations based on standard samples. Furthermore, selected examples from food science will be presented to illustrate the applications this sector.

Keywords: *nanoparticles, electron microscopy, automation, image analysis*

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L69
AN INNOVATIVE CONCEPT TOWARDS STANDARDIZED METHOD DEVELOPMENT TO SEPARATE, CHARACTERIZE AND QUANTIFY ENGINEERED NANOPARTICLES IN FOOD AND COSMETICS

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Generic schemes to systematically develop methods for detection, characterization, and quantification of engineered nanoparticles (ENPs) in complex matrices are required by the industry, by the scientific community as well as by regulatory bodies. Recently, Wagner et al. (2015) published the first tested generic sample preparation scheme for separation, characterization, and quantification of ENPs in complex food matrices which defines quantitative quality criteria. This novel generic sample preparation scheme and the applied quality criteria are of pivotal relevance for standardized method development for ENPs in food and consumer products. In our experimental study we challenged this generic approach and selected a real food sample and a cosmetic sample: sample 1) a powdered tomato soup which contains SiO_2 particles as anti-caking agent (E551), and sample 2) a sunscreen which contains TiO_2 as UV-filter and Fe-oxides as pigment. The two challenges associated with the samples were: A) there was no reference material available for E551 particles in tomato soup, and B) two types of particles (TiO_2 and Fe-oxides) had to be extracted from the sunscreen. Following sample preparation, the extracted particles were analyzed by asymmetric flow field flow fractionation (AF4) online coupled to multi-angle light scattering (MALS) and inductively coupled plasma mass spectrometry (ICPMS). As the most prominent result, it was found that an adapted generic sample preparation scheme in combination with the AF4-MALS–ICPMS analysis is applicable for both matrices. For SiO_2 in tomato soup, complete matrix removal and Si mass recovery > 90% were achieved using acid digestion supported by heat (90°C) and hydrogen peroxide as oxidation agent. The static light scattering signal was used for the first time as a fingerprint to identify the type of particles present in the food matrix. For TiO_2 in sunscreen, ENPs could be isolated by a combination of combustion and solvent washing followed by magnetic separation of TiO_2 and Fe-oxides. Recoveries were sufficiently high to perform FFF-MALS-ICPMS analysis indicating complete separation of TiO_2 and Fe-oxides ENPs. This presentation will demonstrate the application of the generic sample preparation approach in combination with high-end analytical techniques to detect, characterize and quantify ENPs in food and consumer products.

[1] Wagner et al. (2015): J. Anal. At. Spectrom, 2015,30, 1286-1296

Keywords: *sample preparation, engineered nanoparticles, field flow fractionation, light scattering*

Acknowledgement: *Acknowledgment – The NanoDefine project is supported by the European Commission under the Environment (including climate change) Theme of the 7th Framework Program for Research and Technological Development.*

L70
ON-LINE COUPLING OF SIZE SEPARATION BY HDC AND AF4 WITH SPICP-MS FOR IMPROVED ACCURACY IN NANOPARTICLE ANALYSIS

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In recent years successful methods have been developed and applied for the detection and characterization of nanoparticles. These include separation techniques as hydrodynamic chromatography (HDC) and asymmetric flow field flow fractionation (AF4) and characterisation techniques as multi-angle light scattering (MALS) and single particle inductively couple plasma mass spectrometry (spICP–MS). However, even using these techniques it is difficult to discriminate between ions, primary particles and nanoparticle aggregates/agglomerates. Reliable characterization of nanoparticles (NP) and nanoparticle agglomerates is required to assess the fate of nanoparticles in biological and environmental matrices. A large step forward is the on-line combination of separation and characterisation techniques, like HDC-spICP-MS or AF4–spICP–MS. This approach may be suitable to deliver more accurate information regarding the form, size and agglomeration state of nanoparticles. spICP–MS detects and characterises individual nanoparticles at very low concentrations and produces a volume equivalent spherical diameter based on the measured particle mass. HDC and AF4 allow the separation of nanoparticles according to their hydrodynamic diameter, however, usually at higher concentrations. Combinations of HDC and AF4 with spICP–MS have been studied for the detection and characterization of ions, primary nanoparticles and nanoparticle agglomerates. However, combining HDC and AF4 with spICP-MS is not straightforward and possibilities, difficulties and solutions will be discussed. Analytical possibilities have been studied with 10, 30 and 60 nm gold, and 30, 60 and 120 nm silver nanoparticles and gold and silver ions in aqueous samples and in an in-vitro human digestion model. Results will be discussed and show that ions, nanoparticles and nanoparticle agglomerates can be separated with these hyphenated techniques and that dissolution and agglomeration dynami cs can actually be followed in time.

Keywords: *nanoparticles, spICP–MS, on-line coupling, HDC-spICP-MS, AF4–spICP–MS*

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L71*
DETECTION AND CHARACTERIZATION OF ALUMINIUM-CONTAINING NANOPARTICLES IN A COMPLEX FOOD MATRIX

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With the emerging use of nanometer-sized additives in food, there is a need for reliable methods for detection and quantification of nanoparticles (NPs) in complex food matrices in support of risk assessment or future food control. Aluminium (Al) can be found in raw foods as a natural constituent taken up from soil or as a food additive (e.g. anticaking, coloring agents). Depending on its source, it can be present as molecules or as particulates, a fraction of which may occur at the nanometer size scale. Because of relatively high concentration of Al in noodles compared with most other foods, in combination with toxicological concern about the dietary intake of Al, a set of 21 samples of noodles were collected as part of a national monitoring program. The aim of the work was to analyse the total Al content in the noodles, and to develop a method for analysis of Al-containing NPs in this matrix. Firstly, the total Al content of the noodle samples was determin ed by inductively coupled plasma mass spectrometry (ICPMS) following two sample preparation procedures: acid digestion by a mixture of nitric acid and hydrogen peroxide or by a mixture of this acid with hydrofluoric acid (HF). The results demonstrated a consistently higher Al concentration (about 2-fold higher) when HF was used and were 10+/-3 µg/g (range 7–15 µg/g, N=21). This showed that the Al concentration in 11 of 21 samples exceeded the maximum limit of 10 µg/g, and also suggested that some Al was present in compounds only soluble in HF. This led to the hypothesis that Al might be present in the form of particulates (e.g. as aluminium silicate), which were insoluble even in nitric acid. To test this hypothesis the noodles were screened for Al-containing particles by single particle ICPMS (sp-ICPMS) following digestion of the matrix with a mixture of nitric acid and hydrogen peroxide or by enzymatic digestion using amylase. The detection of Al in the digestates by sp - ICPMS (m/z 27 at 3ms dwell time) demonstrated that Al-containing particles were present after both sample preparation procedures, although at a much larger number concentration following enzymatic digestion. The level of dissolved Al and polyatomic background at m/z 27 detected in sp mode was determined by comparing the baseline of a blank sample with that of the digested samples. The number-based size distribution, which was obtained for the Al-containing particles in the enzymatically-digested sample, had a diameter ranging from the lower size limit of detection (49+/-3 nm, N=29) up to approximately 200 nm. Any possible Al-containing NPs below this limit were undetectable by the sp-ICPMS method. The methodology presented is, in a food context, of interest to support the implementation of the draft definition of a nanomaterial anticipated from the European Commission. The results of the study present evidence that Al-containing NPs with a fraction below 100 nm in diameter were present in the investigated samples.

Keywords: *aluminium in food, nanoparticles, single particle ICP–MS, enzymatic digestion, acid digestion*

Acknowledgement: *The Danish Veterinary and Food Administration assisted by providing the investigated samples and by partial funding of the work.*

L72
A SHORT TUTORIAL ON THE POSSIBILITIES AND FUTURE PERSPECTIVES OF ELECTRON MICROSCOPY TECHNIQUES TO MEASURE PARTICLE NUMBER SIZE DISTRIBUTIONS OF NANOMATERIALS

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Nanotechnology (NT) is the art, science and engineering for manipulating objects at the 1–100 nm scale (Nanoparticles (NP)) and opens up a tremendous field of new applications beneficial to mankind and the environment. Amongst others, the food and cosmetics industries have started using NP to increase to quality of their products. However, despite the beneficial use of NP in many products, their interaction with biological systems and ultimately their fate in the environment is still poorly understood. The reason for this lack of knowledge is to a large extent due to our inability to reliably detect any quantify NP, especially in complex matrices. The European Commission's recommendation of the definition of a nanomaterial is based on the particle number size distribution of the respective material and includes "natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm" [1]. Thus, the application / development of analytical techniques that allow establishing particle number size distributions will be crucial from a regulatory perspective. Electron microscopy (EM) in combination with image analysis is promising technique which provides particle number based results. In this tutorial an overview of the possibilities of different electron microscopy techniques, including scanning and transmission electron microscopy, with respect to NP analysis will be given. In addition, a new software tool to automatically extract particle size distributions from recorded EM images, developed within the EU-FP7 project 'NanoDefine', will be presented. However, although EM operations and analysis can largely be automated, the quality of the results will strongly depend on the sample preparation and different approaches to produce optimal samples will be discussed in the second part of this tutorial.

[1] EC (European Commission), Off. J. Eur. Union 2011, 275, 38–40.

Keywords: *nanoparticles, sample preparation, electronmicroscopy*

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L73*
ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION FOR THE DETECTION AND CHARACTERIZATION OF NANOPARTICLES IN FOOD – A SHORT TUTORIAL

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With the increasing use of nanotechnology in food and consumer products, there is a need for reliable detection and characterization methods for nanoparticles (NPs) in complex matrices. NPs often interact with each other or with their surroundings leading to aggregation, adhesion to surfaces or dissolution in dispersion solvents. Accurate and precise characterization of metrics such as size, shape, particle mass and number concentration therefore remains a challenging analytical task. In order to determine quantitative metrics that are relevant in food monitoring or in risk assessment, asymmetric flow field-flow fractionation (AF4) hyphenated with optical detectors and inductively coupled plasma mass spectrometry (ICP–MS) has proven to be a powerful technique [1,2]. Several parameters of the AF4 influence the separation, including carrier liquid composition, membrane material, cross flow rate, spacer height, focus flow rate, focus time and injected mass. In order to acquire accurate data the AF4 separation method must be optimized for each new sample matrix and analyte NP combination [1,3,4]. This tutorial will give guidance for the application of AF4 to the detection and characterization of NPs in food. The most important AF4 separation parameters will be identified and described. The use and relevance of different detection methods, like multi-angle and dynamic light scattering, absorbance, ICP–MS and the new ICP–QQQ–MS, will be presented. Furthermore, the need for suitable sample preparation methods and independent verification of the results, e.g. by transmission electron microscopy (TEM) or single particle ICP–MS, will be highlighted. Finally, the possibility for determination of number-based particle size distribution will be discussed. The determination of a size distribution based on particle number is relevant for future regulatory purposes because of the European Commission's recommendation of the definition of a nanomaterial as a "natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an aggl omerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm" [5].

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Keywords: *nanoparticles, food, field flow fractionation, ICP-MS*

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L74
SINGLE PARTICLE ICP-MS AS A ROUTINE TOOL FOR NANOPARTICAL ANALYSIS. A SHORT TUTORIAL

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Detection and characterization of nanoparticles (NPs) in complex media as consumer products, food and toxicological test media is an essential part of understanding the potential benefits and risks of the application of nanoparticles. Single particle ICP–MS (spICP–MS) has developed quickly as a screening tool for the detection and characterization of nanoparticles in complex matrices. While spICP-MS can be carried out on standard ICP-MS systems and data-processing software is available on the internet, commercial ICP–MS instruments including specific "nano" processing software are becoming available. In spICP-MS, the sample, an aqueous suspension containing the nanoparticles to be analysed, is introduced continuously into an ICP–MS system that acquires data with a high time resolution. Following nebulization, a fraction of the NPs enter the plasma where they are vaporized and the individual atoms ionized resulting in a cloud of ions. This cloud of ions is sampled by the mass spectrometer and detected as a signal pulse in the detector. A typical run time is 60 s and produces a time scan. The number of pulses detected per second is directly proportional to the particle number concentration in the sample while the intensity of the signal pulse is directly proportional to the mass of the detected nanoparticle. Assuming a certain particle shape (e.g, spheres) and composition (i.e, density), one can calculate the volume equivalent spherical diameter of the particle. In this tutorial the basics of spICP–MS will explained, performance characteristics and data processing will be discussed and the applicability of spICP–MS for routine sample analysis will be demonstrated by several examples.

Keywords: *single particle ICP-MS, tutorial, nanoparticle analysis, nanoparticle characterization*

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L75*
COMPARISON OF THE SIZE OF TITANIUM (NANO-) PARTICLES IN SUGAR COATING OF SWEETS OBTAINED BY DLS, AF4–MALLS–ICP–MS AND SP–ICP–MS

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In the last years, nanoparticles (NPs) were found in different consumer products. For example, TiO_2 (E171) and SiO2 (E551) are used as food additives to provide them specific properties. Sizes of the added primary particles of TiO2 and SiO_2 are lower than 100 nm, but generally, these compounds tend to aggregate to large particles of 1,000 nm. European directives intends to regulate the label of foods in this sense that should include the word “nano” if 50% or more of the particles are lower than 100 nm. Furthermore, a specific evaluation of these food additives will be done in December 2015 and 2016. To accomplish these regulations, characterization of NMs becomes now necessary in diverse types of foods. However, for the moment there is no established analytical method to evaluate the presence and the size of the particles in that food samples. This work presents the development of a methodology for the determination of NPs in the sugar-coating of sweets (chocolate candies and chewing gums) where TiO_2 is often added. Firstly, optimization and validation was performed on different types of NPs reference materials for the following techniques: Dynamic Light Scattering (DLS), Asymmetric Flow Field-Flow Fractionation coupled with Multiangle Laser Light Scattering and Inductively-coupled Plasma Mass Spectrometry (AF4–MALLS–ICP–MS) and Single Particle mode Inductively-coupled Plasma Mass Spectrometry (SP-ICP–MS). Then, developed methods were applied to the studied samples. Sample preparation consists in the extraction of TiO_2 contained in the coating with water. From the comparison of the results obtained by the different techniques, DLS gives information of the hydrodynamic diameter but without knowing the composition of the particles. Even if DLS is considered as a simple method, results obtained are in good agreement with most sophisticated techniques. AF4–MALLS also provides the hydrodynamic diameter, as well as the size distribution and the radius of gyration. After its coupling with ICP–MS, composition of the particles of each size can be achieved. SP–ICP–MS allows obtaining simultaneously the size and the composition of the NPs in a faster way than AF4–MALLS–ICP–MS with a similar precision. Depending on the technique employed, the size of the particles contained in the sweet coatings ranges from 80–150 nm, being very close to the size limit given by EU regulation.

Keywords: *nanoparticles, foods, AF4–MALLS, SP–ICP–MS, DLS*

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L76
AMBIENT IONIZATION MASS SPECTROMETRY: TEN YEARS AFTER INTRODUCING DART AND DESI

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In 2015 we are celebrating the 10th anniversary of the two most important techniques from the group of ambient ionization mass spectrometry namely of desorption electrospray (DESI) [1] and direct analysis in real time (DART) [2] which were mentioned for the first time in 2004 and 2005 respectively. In the following years a series of new ionization techniques falling into this sub-group have been developed. Besides DESI with its combined desorption and ionization mechanism and DART, based on the interaction of excited gas molecules (mostly helium) either directly with the analyte or via ionization of solvent molecules with subsequent proton transfer, direct ionization methods have gained increasing interest over the last years [3]. Hereby an ESI process is generated directly from a solid substrate. These direct ionization methods comprise techniques like paper spray -, tissue spray -, wooden tip spray, and thin layer spray mass spectrometry. Thereby direct s pray methods (mostly from solid-substrate ESI methods) show several distinct advantages, including extremely low sample preparation effort, the possibility to directly analyze trace amounts of substances deposited on surfaces without additional extraction step, the avoidance of any clogging problems often encountered in capillary based ESI and the wide range of materials suitable for direct ionization analysis. Not to forget, direct ionization methods can also be seen as a low-cost option to obtain MS spectra from a variety of specimens.

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Keywords: *ambient ionization mass spectrometry, desorption electrospray (DESI), direct analysis in real time (DART)*

L77
AMBIENT MASS SPECTROMETRY IMAGING OF FOOD CONTAMINANTS

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INTRODUCTION
Food contaminants are usually measured in an averaged single dimension only, through extraction and analysis of entire food products or food ingredients. However, levels of contaminants such as pesticides or hazardous natural compounds may vary locally to a large extent, thereby limiting the value of average-based data when particular parts of food (ingredients) are being used and consumed. Also regulatory limits are usually based on entire product analysis data. Laser ablation electrospray ionization (LAESI) mass spectrometry imaging (MSI) does not require very flat surfaces, high precision sample preparation or the addition of matrix. Thanks to these features, LAESI-MSI may be the method of choice for spatially-resolved food contaminant analysis.
METHODS
In this work, LAESI time-of-flight MSI at a mass resolution of 18,000 (FWHM) has been explored for macroscopic and microscopic imaging of pesticides, natural toxins and plant metabolites on rose leaves, oranges, apples, lemons, ergot bodies, cherry tomatoes and maize kernels. The LAESI system was equipped with a 2940 nm mid-IR laser yielding a spot size of 200 µm and the laser was firing ten times per x-y location at 10 Hz. Accurate mass ion map data were acquired at a sampling location center-to-center distance of 0.2-1.0 mm and superimposed onto co-registered optical images.
PRELIMINARY DATA
Spatially-resolved ion maps of pesticides on rose leaves suggest co-application of registered and banned pesticides. Ion maps of the fungicide imazalil show that this compound is only localized on the peel of citrus fruits. However, according to 3D LAESI-MSI the penetration depth of imazalil into the peel shows significant local variations. Ion maps of different plant alkaloids on ergot bodies from rye show co-localization in accordance with expectations. Among them, an untargeted alkaloid was found that has hardly been reported in literature. Untargeted ambient MSI in food analysis is demonstrated by ion maps of plant metabolites in cherry tomatoes and maize kernel slices. In the tomato case, traveling-wave ion mobility (TWIM) was applied to discriminate between different lycoperoside glycoalkaloid isomers; in the maize case quadrupole time-of-flight tandem mass spectrometry (MS/MS) was successfully used to elucidate the structure of a localized unknown.
NOVEL ASPECT
LAESI ambient MSI spatial distributions of a range of targeted and untargeted food contaminants on native sample materials were obtained.

Keywords: *LAESI time-of-flight MSI, food contaminants, plant sample materials*

L78
INNOVATIONS IN FOOD FRAUD DETECTION USING RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY

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The increasing number of reports globally relating to a wide range of food frauds has brought food authenticity and safety to the attention of regulators, industry and consumers worldwide. The recently developed ambient ionization mass spectrometry methods has overcome a number of intrinsic constraints of traditional mass spectrometric analysis schemes, allowing in-situ, real-time analysis of a wide variety of samples. Rapid Evaporative Ionization Mass Spectrometry (REIMS) has been used for the analysis of human and canine tissue during surgery and has shown to be capable of the identification of different tissue types based on the lipid fingerprint of each mass spectrum. In this study, we present for the first time an effective, near real time method to identify fish product speciation and composition using REIMS. A multi center prospective observational study has been designed to test the capability and reproducibility of the REIMS method on fish. Different origin and species of fish have been collected fresh frozen and subjected to analysis. All specimens were sampled using a custom-built monopolar handpiece, that was equipped with a smoke evacuation line connected to an air driven Venturi pump, that was mounted on the atmospheric interface of the Waters Xevo G2-XS mass spectrometer. The full spectral information was recalibrated, normalised, baseline subtracted and binned to 0.1 m/z bin size. The resulting data vectors were subjected to multivariate statistics in order to obtain a classifier for the identification of the species of origin. Principal component analysis (PCA) was used to eliminate chemical noise and reduce the dimensionality of the dataset. Following PCA, the first 25 principal components were subjected to linear discriminant analysis (LDA). The REIMS method has been successfully implemented for fish speciation. In our initial experiments, Atlantic cod, haddock and coley has been sampled and analysed with REIMS method on three different sites. The acquired spectra featured fatty acids in the 150–500 m/z region, glycerophospholipids in the 600-900 region and triglycerides in the 900–1,000 region. PCA loading plots revealed the importance of phosphatidic-acids, phosphatidyl-ethanolamines and phosphatidyl-serines in the separation of Atlantic cod and Alaskan haddock. Leave 20% out cross validation of the PCA followed by LDA models resulted in 94.44% correct classification rate independent from the sampling site and instrument. Application of the REIMS technique for the rapid lipidomic profiling of different fish species was successfully performed for the first time. The described results clearly demonstrate that the lipidomic profiles can be recorded in a few seconds timeframe and can be used for the identification of different fish species. REIMS technology could provide a paradygm shift across many food safety and authenticity applications by providing a real-time, reliable, and simple method for the analysis of food products.

Keywords: *mass spectrometry, food authenticity and fraud, rapid evaporative ionization mass spectrometry*

L79
QUANTITATIVE SCANNING DART-MS SURFACE ANALYSIS FOR DETECTION OF FOOD DYES AND PARABENS AFTER HPTLC, UTLC OR DIRECT BIOAUTOGRAPHIC ASSAY

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Technical modifications for quantitative scanning surface analysis and its progress in performance were demonstrated by coupling high-performance thin-layer chromatography (HPTLC) with Direct Analysis in Real Time mass spectrometry (DART–MS). DART is a novel desorption and ionization technique already hyphenated with gas chromatography and high-performance liquid chromatography [1,2]. Manifold adaptations showed its potential on a wide range of compounds and first attempts for coupling with HPTLC were made [3,4]. The original DART SVP-A-3DS interface was modified for exact positioning and movement of the HPTLC plate, for the exact guidance of the DART gas stream and for enhanced detection performance at reduced mass signal variations [5]. Thus, desorption, ionization and capturing of analytes out of planar substrates were substantially improved. An angled substrate table reduced collisions of the deflected gas stream with the inner sampling tube wall and thus increased detectability. A focused gas stream guidance and low gas scattering after collision with the surface was supported by a shortened source cap with graded inner diameter, a short sampling tube with conical inner diameter and an angled ending towards the substrate. This optimized geometry with open access to the substrate surface and reduced ambient air gap increased efficiency and reproducibility of ion transportation to the MS orifice. MS detection of food dyes after separation on HPTLC and ultrathin-layer chromatography (UTLC) layers was performed and its characteristics were compared [6]. Scanning of butyl-4-hydroxybenzoate applied on the planar substrate showed high precisions and enhanced signal intensities for the optimized source cap and transfer tube geometries. This confirmed the improved desorption efficacy and detectability. After chromatography of methyl-4-hydroxybenzoate and butyl-4-hydroxybenzoate, mean determination coefficients showed quantitative c apabilities. Spatial resolution was determined to be smaller than 1 mm at a scan lane width of 3 mm. Three different direct bioautographic assays were performed prior to DART-MS to estimate the influence of the assay-background on desorption and ionization and thus the opportunity to perform MS detection directly after direct bioautographic tracking of unknown bioactive compounds.

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Keywords: *surface analysis, HPTLC, DART-MS, ambient ionization, effect directed bioautography*

L80
THE CONTRIBUTION OF SYMPHONY PROJECT TO MILK SAFETY

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This paper reports the concepts and the first results of the SYMPHONY project (FP7-ICT-2013-10). The project aims at the sensitive and selective detection of contaminants like aflatoxin M1 and antibiotics in milk with novel technologies for sample preparation and detection. The system will be integrated in the production chain of the dairy industry at acceptance point to provide an effective tool for industry precision, effective use of resources and waste reduction. In this work we will demonstrate the benefits of sample preparation by microfluidic phase separation, immunoaffinity and photonic sensors integration to provide an effective and automated system alternative to lateral flow and ELISA kits for contamination screening and quantification in milk. In food analysis it is often necessary to process the sample before quantification of the chemical analyte to avoid matrix effects. Microfluidics can help in the automation of procedures, leading to greater control and lower work burden on operators. In milk the presence of fat, proteins and many other components in a complex phase equilibrium poses particular challenges for analysis. Casein is usually found in milk in micelles with size of about 50–300 nm, while fat is mostly found in globules in the size range 0.1–20 μm with the prevalent fraction above 1 μm [1]. Microfluidic fat separation can be performed by inertial methods working at a high flow rate. We demonstrated fat separation comparable to centrifugation at flow rates in the range of mls/min. In addition, residual proteins may interact with sensor surfaces and so need to be removed for better accuracy. Since for instance the isoelectric point of casein is 4.6, at milk pH there is an intrinsic negative charge, which can be used to improve the purification of the sample using electric fields in addition to immunoaffinity extraction [2]. High-resolution biosensors, such as SiN asymmetric Mach-Zehnder interferometers (aMZI) and SiON microring resonators (MRR), were selected for analyte detection. Anti-aflatoxin aptamers immobilized on the detector surface are used for the selective detection of aflatoxin, with a demonstrated layer density around 1013 aptamers per cm2. Low cost VCSEL sources and photodiodes are to be integrated on chip by means of heterogeneous assembly. The achieved limit of detection (LOD) was 0.5 10^{-6} Refractive Index Units (RIU) and 1.6 10^{-6} RIU in the case of aMZI and mRR, respectively. The sensor regeneration and reusability was demonstrated up to 17 regeneration cycles.

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Keywords: *microsystems for food analysis, photonic biosensors, microfuidics for sample preparation, aflatoxin M1, milk contaminants*

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L81
FAST, ACCURATE AND QUANTITATIVE DETECTION OF HARMFUL SPECIES IN FOOD AT THE POINT-OF-NEED: THE FOODSNIFFER SOLUTION

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Contamination of food and drinking water by pesticides can pose a serious threat to human health and has therefore to be continuously monitored. EU authorities have determined both the maximum residue limits (MRLs) and the recommended detection methods, usually liquid and gas chromatography combined with mass spectroscopy. These methods provide for the simultaneous determination of a high number of analytes in a single run, with high accuracy and reproducibility. However, the instrumentation required is mainly for laboratory use by highly experienced personnel. Thus it is expected that the availability of small size and easy to operate tools with multi-analyte capabilities would facilitate more systematic food analysis for detection of harmful substances. In the present work, we employed a sensing platform based on White Light Interference Spectroscopy (WLRS) for the simultaneous label-free immunochemical determination of three pesticides, namely chlorpyrifos, thiabendazole and imazalil, in food and drinking water samples. Determination is based on immobilization on spatially distinct areas of a single sensing surface of the respective analyte-protein conjugates. The sensing module is composed by a Si wafer with a 1000 nm-thick SiO_2 layer assembled on a docking station with a microfluidic cell to allow for continuous fluid delivery. Detection is based on illumination of the sensing surface with white light using a reflection probe consisting from a bundle of six fibers at the periphery of probe. As the light beam is reflected on the layers of the sensing element with the different refractive indexes, an interference spectrum is created, collected by the central fiber of the reflection probe and guided to a miniaturized spectro meter. The accumulation of biomolecules onto the sensing surface when mixtures of calibrators or samples with analyte-specific monoclonal antibodies are run over the sensing surface increases the biomolecular adlayer thickness leading to reflected interference spectrum shift. Appropriate signal processing of the reflected spectra obtained in the course of the immunoreaction allows for its monitoring in real-time. The interrogation of the areas corresponding to different analyte is achieved by moving the sensing surface with respect to reflection probe. Using this system, the three targeted pesticides could be determined in less than 30 min with a limit of detection of 40 ng/mL, that is well discriminated from the MRLs set for drinking water (100 ng/mL). The same sensing surface could be used for at least 20 times after regeneration reducing considerably the analysis cost. Due to the small size of the sensing area (3.5 mm^2), the relatively low cost and compact instrumentation and the ability for multi-analyte, label-free and real-time determinations, the proposed sensing system could provide a viable solution for on-site pesticide determinations.

Keywords: *pesticides, label-free detection, simultaneous determination, white light interference spectroscopy*

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L82
DEVELOPMENT OF AN AUTONOMOUS FULLY INTEGRATED SYSTEM FOR BACTERIA DETECTION IN FOOD SAMPLES

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We have developed a fully autonomous analysis system for the detection of pathogens in food samples. To achieve this, bio, nano and micro technologies were converged in order to produce a Lab-ob-Chip (LOC) diagnostic system for sample pre-treatment, target (DNA) amplification and analyte detection. Several innovations were brought together in order to make the final product a scientific as well as economic success. The heart of the system is an acoustic wave sensor based on a Love wave guide configuration. A significant advantage associated with the above device lies in an innovative sensing approach, unique to acoustic systems, which is adopted for label-free nucleic acid sensing. Additional incorporated innovations include a plasma nanotextured module for bacteria capturing, lysis and DNA extraction and a foil-based microPCR module for DNA amplification. The above modules, together with a plastic microfluidic cartridge are assembled on a platform the size of a credit card. All modules as well as the sensor biochips can be fabricated using commercially micro engineering techniques, amenable to large scale production. The proof-of-principle has been demonstrated during the detection of 1 Salmonella bacterium in 25 gr of food sample (milk) in a total of 5 hrs, which includes both the time for a short pre-enrichment step and LOC detection.

Keywords: *food pathogens, biosensors, integrated analysis, lab on a chip, molecular diagnostics*

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L83
BIOFOS: MICRO-RING RESONATOR-BASED BIOPHOTONIC SYSTEM FOR FOOD ANALYSIS

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The aim of this work is to develop a highly selective, highly sensitive Lab-on-a-Chip system for in-situ detection of food contaminations in nuts, olive oil and milk and also for the quantitative detection of lactose in milk. Current methodologies for detection of food contamination, based on heavy analytical tools, cannot guarantee a safe and stable food supply. The need for screening tools that will be still reliable but simple, fast, low-cost, sensitive and portable for in-situ application is thus urgent. BIOFOS aims to address this need through a high-added value, reusable biosensor system based on optical interference and lab-on-a-chip (LoC) technology. The biotechnological platform is based on aptamers which are designed and produced to capture mycotoxins, heavy metals etc. Advanced immobilization processes such as laser based techniques are also discussed in the present manuscript. The transducer is based on micro ring resonators coupled with VCSEL laser sources and Si photodiodes. BIOFOS relies on the ultra-low loss TriPleX photonic platform in order to integrate, on a single chip (4×5 mm^2), 8 micro-ring resonators, a VCSEL and Si photodiodes, and achieve a record detection limit in the change of the refractive index of $5 \cdot 10^{-8}$ RIU. Targeting to a reusable and compact device, BIOFOS relies on the use of aptameric sequences as bio-recognition elements of the sensor, where advanced surface functionalization techniques are used for their immobilization while new microfluidic structures will be introduced for the sample pre-treatment and the regeneration processes. Results on the design and fabrication of the photonic structures and immobilization and regeneration of the aptamers will be discussed in this conference.

Keywords: *micro-ring resonators, aptamer, laser immobilisation process*

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L84*
SIMULTANEOUS LABEL-FREE DETECTION OF THREE ALLERGENS IN RINSING WATER SAMPLES USING AN ARRAY OF MONOLITHICALLY INTEGRATED ON SILICON MACH-ZEHNDER INTERFEROMENTER

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The consumption of contaminated or false-labelled food commodities from people allergic to those foods can pose a serious threat to their health. It is also a burden to the food industry that has to check for possible contamination of different food products manufactured in a common production line through analysis of rinsing water samples collected during its cleaning process. To this end, a portable device for on-site quantitative determinations of such substances in the production floor could be advantageous to currently used laboratory restricted methods. In this work, we present the label-free detection of three allergens, namely, peanut protein, soy protein and gliadin, in a single run using silicon chips with an array of ten silicon nitride waveguide Broad-Band Mach-Zehnder interferometers (BB-MZIs) monolithically integrated along with their respective silicon light-emitting diodes on the same substrate. Each BB-MZI is covered by a SiO_2 cladding layer apart from a 25×600 μm^2 window on the sensing arm which is individually functionalized by spotting with appropriate biomolecules. For the specific application peanut protein, soy protein and gliadin are spotted onto the sensing arm window areas of different BB-MZIs of a single chip to allow for the simultaneous immunochemical determination of these three analytes following a competitive immunoassay format. After biofunctionilization, the chips were blocked, washed and dried under nitrogen stream. An appropriately designed microfluidic cover is then applied and the chips are loaded in the docking station of the measuring apparatus. Thus, for the assay mixtures of specific antibodies against each of the targeted analytes with calibrators containing known amounts of each substance were run over the sensor for 3 min, followed by introduction of secondary antibody solution for other 3 min. The output spectra of each BB-MZI are recorded continuously and subjected to Fourier Tran sform to convert the observed spectral shifts to phase shifts providing the means for real-time signal monitoring. The detection limits achieved were 0.4, 1.25, and 0.1 μg/mL, for peanut protein, soy protein, and gliadin, respectively. These detection limits are below the maximum allowable concentrations of allergens in rinsing water from food production lines. The assays were reproducible with intra- and inter-assay CVs less than 12% and 15%, respectively, and accurate with recoveries ranging from 93 to 110%. Moreover, the chip could be regenerated with 0.1 M glycine/HCl solution and re-used for at least 12 times. Given the short analysis time (6 min), the small chip size, and its excellent analytical performance, the proposed biosensor can be the basis for a portable apparatus aiming to on-site detection of allergens in rinsing water and food samples.

Keywords: *allergens, rinsing water, label-free, simultaneous determination, integrated Mach-Zehnder Interferomenter*

Acknowledgement: *This work was supported by the EU-funded Project "FOODSNIFFER" (FP7-ICT-318319).*

L85
LOW-FIELD PROTON NMR AS A NEW TECHNOLOGY FOR FOOD FRAUD DETECTION

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Bench-top, low-field nuclear magnetic resonance (NMR) spectrometers are a new development in the analytical chemistry toolbox. We report the first results from using a new 60 MHz 1-H NMR bench-top spectrometer, Pulsar, as a screening method to assess food authenticity. In the wake of the 'horsegate' scandal we first trialled this new technology for meat speciation. Low-field NMR generates distinctive triglyceride profiles which reflect the mix of triglyceride fatty acid chains in meat, this being characteristic of each species. Thus 60 MHz 1-H NMR was able to reliably distinguish beef from horse, with early results indicating capacity to also classify other meat types [1]. Similarly we exploited underlying chemical profile differences to characterise edible oils from different types. Again visual inspection was sufficient to distinguish some oil types, whilst more subtle chemical differences were best tackled by careful analysis of the spectra including the use of chemometric methods. For instance in simulated adulteration trials this approach enabled us to detect 11.2% w/w adulteration of hazelnut oil in olive oil - two oils of similar fatty acid composition [2]. For both meat and edible oil the sample preparation is trivial and the d ata acquisition time ~10 minutes, making this comparable to other screening techniques with similar cost such as infrared spectroscopy (FTIR). We compared the performance of 60 MHz 1H NMR to FTIR in the analysis of edible oils and found that low-field proton NMR displays comparable sensitivity and improved specificity. Another food commodity that is thought to suffer from widespread fraud is coffee, through substitution of Arabica with Robusta beans. Here several marker compounds of one or the other species have been identified - diterpenes in particular. HPLC can be used to detect them and is the official method in some countries, although clearly not a rapid screening method. There have also been reports that these compounds produce detectable and isolated signals by high-field NMR [3], opening up the possibility to obtain quick measurements although here the cost is likely to limit routine use. We are currently investigating whether we can translate this to a lower-cost bench-top NMR instrument. In summary, the combination of trivial sample preparation, rapid data acquisition and automated analysis suggests that low-field, high-resolution NMR is a candidate fast screening tool for authenticity testing alongside other spectroscopic techniques. In addition interpretation may soon be further aided by 2-dimensional spectroscopy becoming available for bench-top instruments.

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Keywords: *authenticity, NMR, chemometrics, triglycerides, diterpenes*

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L86
DETERMINATION OF POLYSACCHARIDE GUMS IN GELLED FOOD CONCENTRATES

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Polysaccharides are widely used for structuring food products, e.g. as thickeners in soups, sauces, and seasonings, stabilizers in ice cream and oil-water emulsions, and gelling agents in jams and jellies. The polysaccharides commonly used for structuring are, among others, starches, pectins, alginates, xanthan gum, and plant galactomannans such as guar gum and locust bean gum (LBG), and mixtures thereof. The determination of polysaccharide gums in gelled food concentrates is complicated due to the low gum levels (generally below 1%) and the complex food matrix. Here, we describe tools for the identification and quantification of polysaccharide gums in food concentrates. As an example, the identification and quantification of xanthan gum and LBG present in commercial savory food concentrates is described. It is shown how plant and microbial DNA can be detected in intact food products to demonstrate the use of certain polysaccharides. Other tools include monosaccharide analysis for determining the purity of the polysaccharide fraction after isolation from the food matrix and for the quantification of polysaccharides. NMR spectroscopy is used for identification of the types of polysaccharides as well as for the semi-quantitative determination of mixtures of galactomannans. A newly-developed method based on enzymatic degradation and high-performance anion-exchange chromatography is used for the identification and selective quantification of LBG and other galactomannans. The techniques described give reliable qualitative and quantitative data for the identification and quantification of polysaccharide gums in gelled food concentrates and are therefore ideally suited for e.g. authentication and quality control purposes.

Keywords: *polysaccharide gums, NMR spectroscopy, polymerase chain reaction, enzymatic fingerprinting, monosaccharide analysis*

L87
ANALYTICAL STRATEGY FOR AUTHENTICITY TESTING OF WINE AND TEA SAMPLES USING COMBINED NON-TRADITIONAL STABLE ISOTOPES DETERMINED BY ICP/MC/MS (INDUCTIVE COUPLED PLASMA / MULTICOLLECTION / MASS-SPECTROMETRY)

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Light stable isotope (H, O) and selected non-traditional isotope approaches (Sr, Pb) are already used to determine authenticity of food products. Strontium isotope ratio directly reflects the soil and is widely applied to trace geographic origin, while lead isotopes are extensively used to “fingerprint” important anthropogenic sources. ICP/MC/MS is now recognized as a method of choice for the high precision measurement of non-traditional stable isotope ratios. The benefits of combining information from two isotopic systems to drastically improve the geographic origin and the authenticity of the samples analyzed are presented here. Using two isotopic systems, one tracing the soil (Sr,…), and the other tracing environmental ambient pollution (Pb, Hg,…), now allows exceptional new information to be obtained in terms of traceability. We have applied this new analytical strategy to discriminate with high precision the origin of prestigious wines and tea samples. The determination of strontium and lead isotope abundance ratios by ICP/MC/MS has been applied to 36 samples of Bordeaux wines and 16 samples of Chinese wines. The Sr isotope ratios combined with elemental analysis data for Bordeaux wine samples have demonstrated a clear distinction even between local producers in region of Bordeaux. Lead isotope ratios trend was similar for all French wines and it was significantly different from the group of Chinese wines. Combining two isotopic systems (Sr & Pb) tremendously increases the discrimination possibilities compared to a single isotopic system. This analytical strategy was successfully applied to the task of finding counterfeits in testing group of Bordeaux Grand Cru wines. This strategy was also applied to 36 authentic teas from Japan, China, South Korea, India, Shi Lanka, Vietnam, Turkey and Rwanda. The teas with similar origin have shown specific Sr isotope composition, while only Chinese teas were characterized by the large variability of these values. The distinctive trend of Pb-isotope ratio has been observed for the different geographic regions of sample origin. The same combined isotopic discrimination strategy has also demonstrated drastic discrimination improvements compared to a single isotopic signal. This study demonstrates the new potential of a model based on high-precision determinations using combined non-traditional stable isotopes of Sr and Pb with ICP/MC/MS. Its application can be applied for improved food origin, traceability and authenticity determination. Combining isotope ratios information with elemental analysis data gives a complete tool to detect food frauds, including adulteration of high value products with cheaper substitutes, forgery and falsification.

Keywords: *food authenticity, geographic origin, strontium isotopes, lead isotopes, multicollector ICP–MS*

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L88
RAPID ELEMENTAL ANALYSIS OF FOODS BY USING LASER INDUCED BREAKDOWN SPECTROSCOPY

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In the food industry from the production to consumption, food safety and food quality are important parameters for human health and quality life. For this reason, producers have an attention on rapid and reliable, eco-friendly technologies to follow the food processes. Elemental content of foods provides useful information for authentication of foods, quality control parameter and process follow. In conventional methods atomic absorption spectrometer (AAS), inductively coupled plasma mass spectroscopy (ICP–MS) and X-ray fluorescence (XRF) are used for elemental analysis. These methods are time consuming and need sample preparation step. Laser induced breakdown spectroscopy (LIBS) is a rapid, reliable alternative elemental analysis method and does not require sample preparation step. It is an atomic emission spectroscopy method. System has a high energy laser source which produces a high energy pulse and provides the vaporization of small particles in the sam ple. Formed high temperature spark induces the fractionation of sample to notr, atoms, ions and causes forming of plasma plume. Formed plasma plume above the sample is transmitted to spectrometer via a collected lens. İntensity of atomic spectral lines is correlated with total elemental concentration in the sample. Although LIBS system has a widespread using area, food applications are quite limited. In this study potential of LIBS on food analysis was exhibited by studying with different food groups. In this context Na and NaCl detection in bakery products were performed by using LIBS in a few seconds instead of titration or AAS. Another application was determining of whey adulteration in milk powders to prevent the economic, nutritional and legal implications. Meat is other most adulterated food group. The most applied meat adulteration is addition of cheaper meat species such as pork and horse meat to costlier meat species such as beef. Consumers must be protected from these adulterations because of economical loses, health implications and religious beliefs. In this study chicken, pork and calf meats are discriminated by LIBS and chemometric methods according to mineral component differences. For this reason using LIBS provides rapid and simple analysis to identify meat species compared to conventional methods. LIBS is an elemental analysis method and it is useful for also total mineral content so LIBS was used for ash analysis in flours. Promising results indicates that LIBS ash analysis can be used for other food groups such as meat and fruit juices.

Keywords: *LIBS, milk adulteration, ash analysis, meat adulteration, NaCl measurement*

L89*
87SR/86SR ISOTOPE PATTERN AS A TOOL FOR PROVENANCING OF STURGEON CAVIAR

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Sturgeon caviar is one of the most expensive food commodities in the world. While fish farming is emerging, there is also a decrease in the population due to illegal fishing. As a consequence, sturgeon caviar trade is put under regulation by the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Therefore, analytical tools are required to discriminate farmed from wild caviar, in order to control illegal caviar trade and to foster sustainable farming. While species identification by DNA-based methods are at hand, provenancing of sturgeon caviar is still a challenge. Previous approaches, focusing on the analysis of egg color and size, as well as mRNA, fatty acid and δ15N und δ13C composition, showed that those parameters are not reliable enough for an unambiguous origin determination [1,2]. The $^{87}Sr/^{86}Sr$ isotope ratio reflects the local water chemistry and has been successfully applied for discrimination of wild and hatchery fish [3] as well as authentication of food such as Swedish Kalix-caviar [4]. In this pilot study, untreated caviar as well as processed caviar (i.e. salted) from five sturgeon fish farms in Central Europe (one in Austria and four in Italy) were investigated for their $^{87}Sr/^{86}Sr$ isotopic composition. Water samples were analyzed in order to identify the local isotopic fingerprint, while fish feed was analyzed as a potential additional contributor to the isotopic composition of caviar. Sample preparation was performed according to optimized standard protocols. Samples were measured on a multi collector inductively coupled plasma mass spectrometer (MC ICP-MS) for their $^{87}Sr/^{86}Sr$ isotopic composition. Fish farms from geologically different areas could be differentiated by the isotopic signature of water. Moreover, the signature of these fish farms was different from the water signature of the natural living habitat of the sturgeon in the river Danube. Therefore, a distinction could be accomplished. The information of the natural water habitat was transferred into the fish egg, even though a shift in the isotopic composition by the fish feed used in the fish farms, was observed. Moreover, the impact of the processing of caviar by salting resulted in an isotopic shift. Further investigations will focus on the assessment of the potential contribution of salt, water and fish feed to the isotopic signatures of caviar and multi elemental pattern. Mixing models will be used to evaluate the contribution of different sources to the final isotopic and elemental blend in sturgeon caviar. A fully validated analytical protocol for identifying the original $^{87}Sr/^{86}Sr$ isotope ratio and elemental composition of raw and processed caviar has great potential to act as a new tool in caviar provenancing.

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Keywords: *provenancing, caviar, strontium, isotopes, MC ICP-MS*

L90
MASS SPECTROMETRY DETECTION OF BEEF AND PORK MEAT IN COMPLEX FOOD MATRICES

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In the recent years, food frauds have become a very important issue in the field of food quality and safety. Food frauds include a wide variety of illegal activities, such as dilution with water, replacement of an ingredient with a cheaper one, false geographical origin and so on. The risk of food adulteration is higher in highly processed food, where ingredient substitution can be not immediately detected, and mainly affects high added value foodstuff. For example, frequent adulterations are detected in wine, oil, cheese, fish or meat, as demonstrated by the recent meat scandal in Europe concerning the substitution of beef with horse meat in some sauce preparations and ready to eat food. Also, very common frauds regard also the substitution (totally or in part) of beef with the cheaper pork. Beside quality implications (false declaration on the label), safety problems emerged, in particular about the origin of the adulterating meat. The latter in fact could derive from uncontrolled livestocks, which might be not checked for antibiotics, microbiologic agents and so on. But even when pork is allowed, the accurate quantitation of the exact amount of pork and beef might be challenging. Analytical methods to assess meat composition can be essentially divided into two big groups: DNA-based methods (real time PCR) and protein-based immunochemical methods (ELISA or LFD). Both these methods are very sensitive and specific, but they have some limitations. One of the most important is that in highly processed food DNA is often degraded or hydrolyzed in too short fragments, and protein based methods might also have serious detection problem due to protein denaturation, cross-linking and other side-reactions. Moreover, quantitation with both methods is often very difficult. In the present work, tandem mass spectrometry is presented as an emerging method to detect beef and pork meat in very complex and heavily processed food matrices, such as ragout, both in qualitative than in quantitative way. This food matrix is one of the most difficult to be analyzed, both for the heavy thermal treatment (meat browning and final sterilization) and for the high number of interfering ingredients (tomatoes, vegetables, oil, others). The detection is achieved using different marker peptides, specific for beef and pork meat. In order to concentrate proteins, ragout can be first lyophilized, or the meat pieces can be separated from the other ingredients. Then the proteic fraction is extracted using a solution of urea and thiourea, obtaining myofibrillar proteins. Proteins are then cleaved with proteolytic enzymes and peptides are identified both with high and low resolution mass spectrometry. Once marker peptides are identified, a calibration curve can be constru cted using different percentages of pork and beef meat. The method could be routinely applied to detect adulteration of meat-based preparations.

Keywords: *meat, authenticity, mass spectrometry, marker peptides, food fraud*

L91
HIGH RESOLUTION NMR SPECTROSCOPY APPLIED TO THE FIELD OF ALCOHOLIC DRINK AUTHENTICATION

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NMR spectroscopy is a powerful analytical technology that is widely used in the metabolomics field because of its holistic detection capabilities, quantitative nature, reproducibility and interpretability of spectra. Historically, NMR spectroscopy has been seen as a specialised analytical technology due to the high instrumental costs, dedicated housing facilities, the need for regular supplies of cryogens and for dedicated operators. Recent advances in the technology have resulted in the development of high resolution, low-field benchtop spectrometers. These instruments potentially offer some of the analytical advantages of high field NMR spectroscopy in an average laboratory environment. The application of both high field (500 MHz) and low field (80 MHz) will be presented. In this presentation, NMR spectroscopy is applied to several authenticity issues relevant to the spirits drinks industry from rapid and accurate quantification of alcoholic strength, the detection of industrial denaturants, and multivariate statistical analysis of authentic and fraudulent spirit drinks samples.

Keywords: *NMR, authenticity, spirit drinks*

L92
MICROFLUIDICS PLATFORMS TOWARDS SAMPLE PREPARATION, NUCLEIC ACID IDENTIFICATION AND NEXT GENERATION SEQUENCING FOR ON-SITE APPLICATIONS

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There is an increasing need for on-site applications of DNA-based methods, both, for inspection services, as well as for companies that wish to monitor their supply chain. The capability to apply DNA-based methods in the field, or at 'point-of-use', is critical for the practical application of DNA-based methods where centralised laboratories may not be meet the demand of prompt answers for early intervention, e.g. in logistic chains with short turn-around times. The application fields addressed by DECATHLON are food analysis with specific emphasis on pathogen detection for food safety, GMO identification and customs issues. In recent years much progress has been made in the development of different types of on-site devices that can accommodate DNA-based methods. Within the DECATHLON project two primary approaches for on-site use of DNA-based methods are developed in parallel. The first route is focussed on sample preparation and nucleic acid identification using a centrifugal microfluidic platform. The second focus is the development of a novel sensor, based upon graphene nano-gaps that will be used to detect multiplex targets in processed samples. The centrifugal 'lab-on-a-disc' platform is of increasing interest in the micro-fluidics community particularly for low-cost, rugged point-of-use applications such as medical diagnostics and environmental monitoring. This paper will present results taken from a 'DNA-to-Answer' cartridge. This platform, for the first time, will demonstrate the spatially multiplexed detection of a DNA sample by discretising a sample into 12 individual wells. Combined, using valving, with LAMP reagents and primers, this disc is capable of addressing 12 different gene targets; towards identification of Shiga Toxin producing E. Coli (STEC) serotypes. This approach, compared with fluorescent multiplexing, simplifies the reagent assays and the required fluorescent instrumentation; thus contributing to the ruggedness of the platform. Nanogap detectors are very promising for genomic screening, in particular DNA sequencing. Graphene nanogaps (single atom thick), can – in principle – achieve single nucleotide resolution (only one nucleotide inside the gap vs 100 nucleotides inside the conventional ~30 nm thick solid state pores). Nanogaps can be patterned in a freestanding monolayer of graphene with high precision using TEM lithography. However the free standing membrane is made unstable and brittle in the process of patterning electrodes (which is necessary for electrical detection). Membrane tension can induce tearing of the graphene upon liquid incubation and therefore result in subsequent folding and rupturing of the freestanding structures. This section will present the investigation of alternative fabrication protocols. This represents the first steps towards integrating a graphene based sensor onto a point-of-use food analysis platform for next generation sequencing.

Keywords: *on-site application, centrifugal microfluidics, sample preparation, graphene, next generation sequencing*

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L93
THE ASSESSMENT OF LAMP ASSAYS FOR SPECIES IDENTIFICATION IN FOODSTUFF

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The 2013 European incident with undeclared horse meat made it clear that governmental enforcement tasks carried out by food inspectors would strongly profit from the availability of a specific and sensitive on-site detection method for horse meat that is easy to perform, fast, robust and cost efficient. Specific detection of horse DNA by loop-mediated isothermal amplification (LAMP) seems one of the most promising methods to meet these criteria. In the present study an assessment of the specificity and sensitivity of the LAMP horse screening assay was made and outcomes were compared with the EURL-AP (European Reference laboratory for animal proteins) qPCR method. The specificity was tested with DNA samples from seven different species, i.e. next to horse, also buffalo, chicken, turkey, pig, grasshopper, deer and bovine. The sensitivity of the LAMP assay was subsequently challenged with different percentages of horse DNA in bovine DNA and different percentages of horse meat in beef. Showing that the LAMP method is both specific and sensitive. Next, 69 processed meat samples were screened for horse. The results showed that the LAMP horse assay, combined with a simple and fast DNA extraction, results in similar outcomes as the EURL-AP qPCR method and is thus a promising screening assay to be used on-site. Only samples that are screened on-site as suspect in the LAMP horse assay, should be brought to the laboratory for confirmation with the more laborious and expensive EURL-AP qPCR reference method. Finally, a start was made with the evaluation of several available LAMP assays for the identification of other species, i.e. bovine, chicken and pig, in meat and processed food products. Binary mixtures of meat in other meat species were prepared to assess the detection limit of the assays. Just as for horse, both qPCR and LAMP were able to reliably detect as low as 0.1% target material. However, late signals were observed for several closely-related species in these LAMP assays, i.e. turkey in the LAMP chicken assay and buffalo in the LAMP bovine assay, emphasizing the need to set cut-off values, similar as in qPCR methods. Overall, LAMP assays were able to give reliable results for the direct identification of horse, chicken, pig, and cattle in under 20 minutes, and could provide a simple, quick, and robust on-site detection method for food inspectors.

Keywords: *fraud, horse, detection, on-site, quick*

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L94
METABARCODING – THE NEXT GENERATION IN SPECIES BARCODING

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For authenticity control of food and feed samples, all parts of the "foodomics" cascade are targeted by analytical methods. Whereas several years ago mainly targeted analytical methods were used in the genomics, proteomics as well as metabolomics fields, in the last years non-targeted screening methods became more and more popular. This trend is due to new method developments and new instrumental setups that can be combined with bioinformatical tools and thus enable evaluation of huge data amounts obtained in one single run. DNA barcoding was the beginning of non-targeted DNA-based species analysis and is now state of the art for animal, plant and microbial species identification in food and environmental samples with unknown composition. However, the identification of species in complex products by this approach is challenging due to the overlay of the sequences of all species in the Sanger chromatogram. The use of next generation sequencing (NGS) approaches enables massive parallel but individual barcode sequencing of all species sequences present in one sample (= metabarcoding). Additionally, multiplexing of hundreds of samples in one run is possible. This makes NGS approaches to a perfect tool for non-targeted screening of food and feed samples with respect to species identification. Hence, metabarcoding can be used for animal as well as plant species authenticity control in food and feed samples. Apart from that, microbiota analysis by NGS can be used for monitoring of fermentation processes and ripening stages and for pathogen detection. The oral presentation will give an overview on the ongoing developments at Eurofins.

Keywords: *authenticity, fraud, non-targeted analysis, next generation sequencing, metabarcoding*

L95
PHENOTYPING OF BACTERIAL COLONIES FROM THE BIOPHOTONICS PERSPECTIVE: THE FUNDAMENTALS OF PATHOGEN DETECTION

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Detecting and identifying food borne pathogens is a critical aspect of food safety. A new technology for achieving this uses laser light scatter in a label-free technology. A bacterial colony is a cluster of individual cells that are organized as a multicellular entity. Historically, bacterial cells are regarded as unicellular organisms, but when they expand into colonies they start to form complex communities with specific divisions of function and population differentiation. Recent development of forward scattering phenotyping instrument had revealed remarkable resolving power of discriminating genera/species and some strains without destroying or labeling the samples. This phenomenon is the accumulative effect of the photons and cell interactions which encodes the minute differences in micro- and macroscopic structure and biochemical materials of each species of bacteria. We present the theoretical modeling of bacterial colony as an optical phase modul ator. Scalar diffraction theory models the colony as a amplitude and phase modulator. To provide the insight of correlations between micro-scale morphologies to the scattering phenomena, various different measurement modalities (phase contrast microscope (PCM), scanning electron microscope (SEM), and confocal displacement meters (CDM)) were utilized.

Keywords: *light scattering, bacterial colony, scatterometry*

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L96
TACKLING THE ILLEGAL ADMINISTRATION OF GROWTH-PROMOTERS IN FOOD PRODUCING ANIMALS: RESULTS AND PERSPECTIVES OF THE HISTOPATHOLOGICAL APPROACH

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It is well known that growth promoters (i.e. sex steroids, glucocorticoids and β-agonists) are illegally used in livestock. The administration of such substances is regulated by the EC Reg. 37/2010 and Dir .96/22/EC, because, besides the positive effects exerted on the muscles of the treated animals, they can accumulate as residues in the edible tissues with severe acute or long-term effects on human health. Despite the EU ban and the high frequency of official controls, there are several cases of illegal administration of such compounds. The results of the European Residues Control Plan show that the issue related to illegal administration is negligible (less than 0.01% of non-compliant samples), but the true extent of the illegal use of growth-promoters may be underestimated. This is probably due to the evolution of the illegal strategy: both the administration of low-dose drug cocktail (estrogens and glucocorticoids or β-agonists) or endogenous compounds (i.e. 17β-estradiol or testosterone) make the residues detection very difficult. The effective enforcement of the ban on growth-promoters misuse requires an increase in knowledge about their metabolism, to determine the physiological concentrations of endogenous substances in different matrices. In this regard, the European Food Safety Authority (EFSA), in a recent opinion "Scientific opinion on the public health hazards to be covered by inspection of meat (bovine animals)", identifies the weaknesses of the official analytical methods for residues control and states the need for new detection strategies, shifting the target from the identification of the molecule/metabolite towards the highlighting of their biological effects. All this considered, this presentation is focused on the results and the recent advances in the implementation of the histopathological approach in the detection of the microscopical lesions induced by growth promoters in calves' target organs.

Keywords: *illicit, treatment, histology, anabolic, growth promoter*

L97
EFFICIENT AND COST-EFFECTIVE BIOANALYTICAL SCREENING OF FOOD SAMPLES FOR ELEVATED LEVELS OF DIOXINS AND PCBS ACCORDING TO THE REQUIREMENTS SET OUT IN COMMISSION REGULATION (EU) NO 589/2014

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Since their development in the 1990s, cell-based bioanalytical screening methods have been effectively applied in environmental and toxicological research laboratories and in laboratories for official control. Certain legal criteria apply when checking samples for elevated levels of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like PCBs (DL-PCBs) within official control of feed and food. These criteria were evaluated by experts from the EU-RL for Dioxins and PCBs in Feed and Food and from National Reference Laboratories (NRLs) in 2010/2011, adopted by EU-legislation in 2012, and amended in 2014. An initial validation process, cut-off concentrations, stringent run acceptance criteria and on-going quality control based on confirmatory methods (gas chromatography/high resolution mass spectrometry (GC/HRMS) or gas chromatography/tandem mass spectrometry (GC–MS/MS)), make up key aspects of the new requirements. The extent of correspondence of Bioanalytical results expressed as Bioanalytical EQuivalents (BEQs) to Toxic Equivalents (TEQs) in which EU regulatory limits are given is an essential outcome of validation and QC processes and must be evaluated from matrix-matched calibration experiments. BEQ-based cut-offs ensuring false compliant rates <5% are derived, above which a sample is declared to be suspected to exceed the respective EU maximum and/or action levels, requiring follow-up confirmatory analysis. This concept requires close co-operation between the two partner-labs and may considerably reduce the GC/HRMS or GC–MS/MS lab's workload by sieving out compliant samples.
Within the scope of establishing strong EU-wide standards for routine and reference methods, the EU-RL has evaluated the performance of various Chemically Activated LUciferase gene eXpression (CALUX) bioassays available on the European Market. CALUX detects 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and structurally related halogenated aromatic hydrocarbons (HAHs) based on their ability to activate the aryl hydrocarbon receptor (AhR) signalling pathway. Some bioassays, however, failed to meet all aspects of the legal requirements. Therefore, the EU-RL has optimized and/or partially re-developed, and validated, CALUX bioassays for 21 target analyte group/food sample-of-interest combinations:
1. PCDD/F-BEQs and DL-PCB-BEQs: bovine fat and meat, pork, bovine and sheep liver, fish oil, hen's eggs, cow's milk and milk fat, human milk
2. PCDD/F-PCB-BEQs: bovine fat, bovine meat, bovine liver, sheep liver, fish muscle tissue, fish oil, hen's eggs, cow's milk and milk fat, human milk, vegetable oil
The new legal requirements prove beneficial to the analyst and represent a strong driving force for thorough step-by-step method optimization. They also provide an effective framework for achieving a high level of performance in bioanalytical screening as demonstrated at EU-RL by internal quality control and frequent successful participation in PT studies.

Keywords: *bioassay, dioxins and PCBs, food, validation, EU maximum levels*

Acknowledgement: *The European Commission's funding of this study is gratefully acknowledged.*

L98*
BIO-FUNCTIONALIZED NANOPARTICLES AS NOVEL BIO-REACTIVE SURFACES FOR THE IMMUNOSENSING OF STAPHYLOCOCCAL ENTEROTOXIN B BY ANODIC IMMUNO-STRIPPING MECHANISM

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Recent trends in biosensing have paved the way for nanoparticle based electroanalytical approach as a promising sensitive technique. Herein, we report carbon nanosphere (CNS) functioned SEB binding DNA aptamer as a novel bio-reactive surface in the presence of anti-SEB antibody functionalized CdTe quantum dot (QD) as detector probe. This sandwich approach has been made use for the immunosensing of enterotoxin B producing Staphylococcus aureus by anodic immuno-stripping mechanism. CdTe-QD of ~2.6 nm having a band gap of 2.46 eV and CNS of ~15 nm were synthesized by colloidal route. The size of the water-dispersible CNS was precisely controlled by ultrasonication (40 kHz) followed by step-wise ultracentrifugation up to 157,000 g. Structural analysis was carried out by absorption, fluorescent and IR spectroscopy. Transmission Electron Microscopy and Atomic Force Microscopy established the spherical morphology of both NPs. Zeta potential distributions were -43.5 mV and -28.8 mV respectively, for CdTe-QD and CNS. CdTe QD was bioconjugated with anti-SEB antibody and characterized to quantify the loading of nanoparticle. The bursting of QD released 4.78 nmoles of cadmium in 1 microlitre (1 micro molar) solution, whereas, the anti-SEB-CdTe QD nanobioprobe bursting resulted in the release of 17.29 nmoles of cadmium per 1 microgram of antibody. Further, a novel bio-reactive platform was developed by modifying glassy carbon electrode (GCE) with CNS electrodeposited through fast scan linear sweep voltametry. Sandwich immunoassay was realized in the presence of SEB binding DNA aptamer coupled with CdTe-QD as detector probe. Oxidation of cadmium was recorded at -0.8 V using square wave voltametry after bursting CdTe-QDs. There was a 5 fold increase in the current as a result of CNS functionalization compared to bare GCE surface indicating enhanced electrode efficiency. The experimental observations suggest the role of CNS in providing an enh anced working area and electrode efficiency. These initial results provided an insight into the governing factors of immunostripping inferring the potency of biofunctionalized semiconductor/inorganic nanodots for electroanalytical applications.

Keywords: *nano-biotechnology, biosensors, staphylococcal enterotoxin B, carbon nanosphere, aptamers*

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L99
NEW DEVELOPMENTS IN THE MONITORING OF CHEMICAL RESIDUES IN FOOD

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As technology continues to improve and world food trade increases, we are investigating new ways to meet needs for high-throughput monitoring and achieve high quality results of a wide array of chemicals of regulatory concern in all types of food. This presentation is intended to describe new developments to overcome weak links and bottlenecks in the overall analytical process from sample processing to reporting of results. In recent years, improvements in sample preparation (extraction and cleanup) and analytical separations (fast chromatography and mass spectrometry with high selectivity and sensitivity) have enabled simultaneous quantification and identification of hundreds of analytes at regulatory levels in foods in batches of >50 samples in <8 hours. These steps can be linked and automated using current robotic tools and instruments. However, the steps of sample processing and data review for so many samples and analytes can each take days of human labor prior and after the “high-throughput” analyses. In the case of sample processing, new bulk comminution devices are needed to work in parallel, not sequentially. For analyte peak integration, identification, and quantification, an automatic software-based approach is needed that does not require human review to yield accurate results (true and precise concentrations without false positives or negatives). I believe this is best achieved by using the summation integration function at expected retention times and peak widths for targeted analytes, including forced integration of noise, which is followed by post-data processing of results. Only identified analytes are reported when pre-defined mass spectrometric criteria and reporting concentration limits are met. Human review of reported positives can be quick and easy, and all suspected violations must be confirmed via re-analysis (including extraction of a duplicate test portion) using an orthogonally-selective method. Other new developments to be discussed include flow-injection or isocratic fast liquid chromatography (LC) paired with tandem mass spectrometry (MS/MS) to conduct analyses for >100 veterinary drug and/or pesticide analytes in <3 min; use of a new, unique cleanup sorbent for “enhanced matrix removal” of lipids and other components in fatty samples, without loss of veterinary drugs, pesticides, or environmental contaminants; and development and validation of of an improved rapid LC–MS/MS method to analyze highly polar aminoglycosides at the same time as a diverse range of other veterinary drug residues in food animal tissues. These developments are continuing the trend of “faster, better, and more” that has greatly improved chemical residue monitoring of foods compared with the past.

Keywords: *veterinary drugs, pesticides, environmental contaminants, monitoring, method validation*

L100
UNDERSTANDING THE POTENTIAL IMPACT OF MILK PROCESSING ON THE DISTRIBUTION OF POPS RESIDUES IN MILK PRODUCTS

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It has long been acknowledged that human exposure to persistent organic pollutants (POPs) occurs principally through our food. Therefore, market basket surveys of the food supply have been valuable tools to provide estimates of human exposures to POPs and the risks associated with these exposures. However, very little is known about how POPs may be distributed in food products during processing events. It would be a safe assumption that lipophilic compounds, such as POPs, would distribute into lipophilic compartments during processing if such compartments are available. Thus, a contaminant's log Kow may be a valuable predictor when modeling the behavior of contaminants in aqueous:lipid mixtures. However, these types of data have not been generated for POPs that may be present in milk, and therefore, it is not known if milk, a complex matrix consisting of fat globules, proteins of various molecular weights, sugars, and water, can be treated simply as an aqueous:lipid mixture. Data will be presented on how a representative dioxin, PCB, and two brominated flame retardants partition into skim milk, milk fat, curd, whey, and concentrated whey proteins (retentate) in a lab-scale simulation of whole milk processing Four 14C-labeled POPs, 1,2,7,8-tetrachlorodibenzo-p-dioxin (TCDD); 2,3',4,4',5-pentachlorobiphenyl (PCB 118); ß-hexabromocyclododecane (HBCDD); and tetrabromobisphenol-A (TBBP-A) were used for this study. Greater than 80% of the fortified TCDD, PCB-118, and β-HBCDD distributed into the milk fat fraction. Specifically, milk fat (and skim milk) consisted of 84.2% ± 3.6 (6.9% ± 1.7), 83.5% ± 3.4 (4.6% ± 0.7), and 87.2% ± 2.0 (3.5 ± 0.4%) of TCDD, PCB 118, and HBCDD after a 30 min incubation, respectively. However, TBBP-A distributed evenly between milk fat and skim milk, i.e. 46.4 ± 0.8% and 45.3 ± 1.3%, respectively. Skim milk was subjected to curding processes, and ≥85% of the remaining contaminants concentrated in the curd while 10-15% partitioned into the whey fraction. TBBP-A was an exception since 45% of the remaining dose distributed to the curd and 55% was in the whey. Ultrafiltration of the whey resulted in ≥85% of the remaining contaminants conc entrating in whey protein retentate for all POPs used. The POPs chosen for the study had log Kow values ranging from 6.8–7.58 (TCDD), 3.2–6.4 (TBBP-A), 5.12–6.6 (HBCDD), and 7.12 (PCB-118) and these values were used to evaluate the contaminant distribution between milk fat and skim milk compared to the log ([POP]milk fat/[POP]skim milk). From preliminary results, log Kow alone does not explain the distribution of these contaminants into specific milk fractions. Furthermore, depending on the POP, milk fraction distribution data indicated that processing can result in contaminant concentration increases in finished milk products.

Keywords: *POPs, milk products, distribution, log Kow, environmental contaminants*

L101
DISTRIBUTION AND CHEMICAL FATE OF CHLORINE DIOXIDE GAS DURING SANITATION OF TOMATOES AND CANTALOUPE

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Although the efficacy of chlorine dioxide gas (ClO_2) against *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella enterica* is well established, the use of gaseous ClO_2 on produce destined for human consumption is not authorized by US regulatory agencies. The major obstacle precluding regulatory approval has been the lack of fate and residue data associated with produce sanitization. Therefore, a series of studies was conducted to establish the 1) distribution and chemical fate of $^{36}ClO_2$ on tomatoes and cantaloupe; and 2) the magnitude of residues in kilogram quantities of tomatoes and cantaloupe sanitized with a slow-release chlorine dioxide formulation. Tomatoes and cantaloupe were respectively treated with 50 and 100 mg – per kg of mass for 2-h durations in sealed glass containers exposed to light. Radioactive residues were concentrated on reaction vessel walls and on the stem-scar area of tomatoes, representing 25–36% of the total $^{36}ClO_2$ generated; cantaloupe rind contained 52% of the total $36ClO_2$ produced. Edible flesh of cantaloupe did not contain detectable radioactive residues. Radioactivity in tomato tank rinses was composed of ^{36}Cl-chloride, ^{36}Cl-chlorate, and ^{36}Cl-perchlorate with the same ions occurring in tomato serum, but with ^{36}Cl-chloride predominating (>80%). In cantaloupe, radioactive residues were not detectable in edible flesh and insufficient residue was present in seed bed for speciation. Cantaloupe rind contained mostly chloride ion, with lesser amounts of chlorate and with perchlorate occurring just above detection levels. Chlorite ion (ClO_2^-) was not present in rinse fractions, or in tomato or can taloupe homogenates. ^{36}Cl-Chlorite added to tomatoes at high levels (131 µg/g) was completely transformed to ^{36}Cl-chloride (98.3%) and ^{36}Cl-chlorate (1.7%). Subsequent studies with unlabeled ClO_2 gas unequivocally demonstrated that the major variable influencing the formation of perchlorate and chlorate during ClO_2 generation was the presence of light. Therefore, kilogram quantities of tomatoes and cantaloupes were sanitized with 50 and 100 mg ClO_2, respectively, and residues determined in edible and inedible fractions using a slow-release ClO_2 formulation. Sanitation experiments were conducted in the dark and residues in homogenates were quantified using LC–MS-MS methods. Chlorate residues were not quantifiable (LOQ, 60 ng/g) in control or treated tomatoes and perchlorate residues (9 ng/g) were similar ($P > 0.05$) in control and treated groups. In cantaloupe edible flesh, chlorate residues were not detectable (LOD 30 ng/g) and perchlorate residues were below the LOQ (1.5 ng/g); cantaloupe rind + edible flesh, however contained 1300 ± 250 ng/g of chlorate with perchlorate residues (2.2 ± 0.2 ng/g) being similar ($P > 0.05$) to con trol cantaloupe (1.9 ± 0.03). Collectively these results suggest that slow-release ClO_2 gas could be a safe and effective tool to prevent the contamination of produce with pathogenic bacteria.

Keywords: *chlorine dioxide, residue, tomato, cantaloupe, chloroxyanion*

Acknowledgement*: ICA TriNova*

L102
OPTICAL METHODS FOR RAPID DETECTION OF PATHOGENS AND FOREIGN MATERIAL IN POULTRY AND FOOD

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While methods for early detection of pathogens are being developed around the world, there are still needs for improvements in speed, sensitivity, and selectivity. Research in the Quality and Safety Assessment Research Unit, USDA ARS in Athens, Georgia is utilizing numerous optical detection methods to rapidly detect both pathogens and foreign material. Research is primarily focused around a core of spectroscopy and hyperspectral imaging. Scientists are exploring means to identify single pathogenic bacteria with hyperspectral microscopy. Protocols have been developed to immobilize live cells so that hyperspectral data of single cells can be collected. Spectral signature (450 – 800 nm), along with shape features, are being combined to classify five Salmonella serotypes. Using only spectral information, classification with a Support Vector Machine algorithms has resulted in an initial accuracy of 84%. However, accuracy is expected to increase as spatia l information is added to the model. To further speed the classification process of single cells, a method to automate cell counting, based on hyperspectral images, was also developed. The technique identifies the optimal single band for colony segmentation and utilizes an automated intensity threshold and separation algorithm resulting in an overall 99% accuracy. The method was also extended to image analysis of fluorescence in situ hybridization where the algorithm outperformed several of the commercially available automated cell counting systems. In addition to identifying and counting single bacterial, hyperspectral imaging systems have also been used in the near field to detect foreign material in a flowing stream of food material. The hyperspectral imaging system, operating between 400 to 900 nm, was based on a universal design originally developed in USDA–ARS for detecting fecal and diseased poultry carcasses. First spectral libraries of dried fruits were collected along with many materials found in the fruit stream that are classified as foreign materials, such as stems, sticks, rocks, plastics, and metals. Then an algorithm, utilizing three to five wavelengths, was developed and implement within the architecture of the universal hyperspectral imaging system. The algorithm was next loaded into the system and a few calibration images were collected before the system started capturing images. The hyperspectral imaging system was able to capture up to 600 frames per second and was easily able to scan, collec t data, calibrate the data, and then identify foreign material all within the constraints of the moving product stream. Finally the hyperspectral imaging system was interfaced with a robotic device to remove any foreign material from the product stream.

Keywords: *hyperspectral imaging, spectroscopy*

L103
A UNIVERSAL ASSAY FOR DETECTING SHIGA TOXIN-PRODUCING E. COLI AND ITS USE IN ANALYSIS OF BACTERIAL CONTAMINATION IN GROUND BEEF

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Shiga toxin-producing *E. coli* (STEC) is one of the most commonly heard pathogen in the news in associated with foodborne outbreaks. Because of its high pathogenicity in humans, these pathogens have rapidly become a major concern. Reliable methods for detection of STEC are crucial. *Shiga toxin* (Stx) as a common trait of all STEC is considered one of the most reliable targets for diagnosis of STEC and several commercial kits have been developed. However, no universal assay effective for all subtypes of Stxs is known so far. To close this gap, we developed new monoclonal and polyclonal antibodies against Stx1 and Stx2 and established an ELISA, which detects all three subtypes of Stx1 (Stx1a, Stx1c, and Stx1d) and seven subtypes of Stx2 (Stx2a through Stx2g) by incorporating newly developed high affinity antibodies in the assay. In order to accurately evaluate the assay sensitivity, standards for each subtype of Stxs were generated. The new ELISA was demo nstrated to be highly sensitive with limit of detection (LOD) ranging between 10 and 50 pg/mL for different subtypes of Stxs. Identification of STEC was able to perform on the supernatants of culture fluids and single colonies of STEC on agar plates were distinguishable based on the production of Stxs without lengthy enrichment in liquid medium. Best of all, this assay was capable of detecting Stx in ground beef spiked with as low as a single STEC cell, following an enrichment. This study significantly improved the current technologies by avoiding false negative results due to the narrow detection range of assays based on the production of Stxs. The assay developed in this study could be useful for identification of outbreaks, potential sources of contamination and prevent further transmission of STEC from the sources.

Keywords: *shiga toxin-producing E. coli, shiga toxin, antibody production, enzyme-linked immunosorbent assay, ground beef*

L104
VOLATILES FROM DEVELOPING FUNGAL SPORES AS EARLY WARNING SIGNALS OF FUNGAL CONTAMINATION AND THEIR DETECTION BY PORTABLE GC-MS SYSTEMS

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Aflatoxin contamination of almonds and pistachios results in millions of dollars of lost product annually. Aflatoxins are metabolites produced by *Aspergillus flavus* and *A. parasiticus*, ubiquitous fungi of California tree nut orchards, and represent a grave food safety problem due to their carcinogenic attributes. Current methods of analysis involve removal and destruction of 20 kilograms of product, utilize laboratory-based methods (e.g. HPLC), are expensive, and can require weeks for results. Because aflatoxin contamination can be limited to one- or two-highly contaminated nuts, current methods do not guarantee detection of the infected nuts, thus posing serious health and safety concerns for the public. Despite an increase in self-regulation and aflatoxin testing protocols by the almond and pistachio industry, the costly rejection of exported product continues. A reliable, early warning aspergilli detector has been cited as a critical need of the industry. Recent investigations have determined that fungal spores emit distinct volatiles when transitioning from the resting stage to germination. Discussed will be the use of volatile profiles to distinguish between contaminated and non-contaminated almond and pistachio samples, and the use of a near real time portable field volatile sampling device to provide early warning detection of fungal-infected "hot spots" from stockpiles and transit containers. Detection and removal of these fungal hot spots decreases human health risks and product loss resulting from Aspergillus contamination.

Keywords: *aflatoxin, Aspergillus, almond, pistachio, MVOC*

TUTORIAL ON 'DATA QUALITY AND SMART DATA HANDLING IN FOOD ANALYSIS'

L105
DATA PROCESSING AND IDENTIFICATION OF SMALL MOLECULES IN LC–MS-BASED NON-TARGETED ANALYSIS WORKFLOWS

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Presentation will focus on:

- Overview of non-targeted workflows in food analysis
  (Workflows and terminology)
- LC-MS platforms and approaches to non-targeted data acquisition
- Requirements and assurance of HR-MS data quality
  (Mass locking, re-calibration of mass spectra, RT normalization, internal standards,…)
- Export of data for chemometric handling
- Data mining, pre-processing and analysis
  (Tools, methods, (un)supervised pattern recognition,…)
- Identification of small molecules: approaches and tools
  (Elemental formula generation, mass spectral libraries and chemical databases, prediction and interpretation of mass spectra,…)
- Examples
- General recommendations

L106
CRITICAL REVIEW, EXPERIENCES AND OUTLOOK WITH RESPECT TO METABOLOMICS DATA HANDLING OPTIONS

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Presentation will focus on key parameters in ensuring quality of LC-HRMS metabolomics data and models

- Overview of LC-MS based metabolomics workflow
  (Identification of critical steps)
- Quality of the samples
  (Key parameters in designing the experiment)
- Analytical Quality
  (Expectations and requirements in both sample preparation and fingerprinting steps)
- Data Analysis Quality
  (Tools for validating the models / markers)
- Illustrations and General recommendations to ensure robustness of the whole workflow

L107
FOOD PACKAGING MATERIAL AND THE INTERACTION WITH THE PACKED GOOD AND THE ANALYTICAL CHALLENGES

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It is a fundamental requirement of all legal bodies as well as of any hygiene management system (e.g. DIN EN 15593 2008, ISO22000 or ISO/TS 22002, BRC/IoP, IFS…) that there is no transfer of chemicals into the packed good which endanger the end user at the point of sale under normal or foreseeable conditions of use. The Regulation (EC) No. 1935/2004 "on materials and articles intended to come into contact with food" defines the requirements for all food contact materials in general. The European Framework Regulation (EC) No. 1935/2004 includes all material and articles intended to come into contact with food. This regulation, Regulation (EC) No. 1935/2004 covers not only packaging material (plastic, paper, metal,…) but also converting machines, pipes, bond-conveyors, tanks, cutting boards and other kitchen wear etc. It declares that any article intended to come into contact with food must be sufficiently inert to preclude substances from being transferred to food in quantities that may endanger human health or to bring about an unacceptable change in the composition of the food or deterioration in its organoleptic properties [1]. However food contact materials contain many substances that can migrate into the food, therefor the Regulation (EC) No. 1935/2004 is accompanied by specific measures for controlling the legal provisions depending on the type of the food contact material. Beside many "traditional" analytes (like heavy metals, PCP, PCB, PAH, etc.) especially UV stabilizers, photosensitizers for printing inks and varnishes (e.g. derivates of benzophenones), dyes (e.g. primary aromatic amines), and endocrine disruptors (bisphenol A, phthalates, etc.) are actually intensely discussed in any food contact application and their impact to the consumers [2,3]. Besides the above mentioned substances, unknown substances can enter the process chain and must be identified and quantified to ensure the product quality (Non-Intentionally Added Substances, NIAS) [4]. NIAS (as defined in the Regulation (EC) No. 10/2011 – plastic implementation measure) are impurities in the substances used for manufacture or reaction intermediates formed during the production process or decomposition or reaction products occurring in the final product. Generally, it is accepted that only compounds <1,000 Dalton are considered as NIAS, because substances with a higher molecular weight are regarded as inert towards migration due to their larger size. Very often the volatile composition can be complex and therefore demands sensitive and selective methods. The combination of gas chromatographic separation with mass spectrometric detection is still the gold standard. The optimization process for analytical methods is always a compromise of speed, selectivity, sensitivity and cost. A proper analytical process has optimized all three relevant parts (sample preparation, separation and detection). It should be kept in mind that there are no ideal sample preparations and measuring instruments available at the market, so each method should be checked carefully if the instrumentation and the methods fulfill the requirements in terms of sensitivity, reproducibility and accuracy. Another problem can be the identification of odorous volatiles, because here no legal limits exist (except in cases of specific migration limits). The limiting factor is given by the sensitivity of the human nose and therefore detection limits in the nanogram per kilogram limit or even lower must be reached. Several examples of analytical strategies will be discussed in this presentation.

[1] European Commission, Regulation (EC) No. 1935/2004 of the European Parliament and the Council of 27 October 2004 on materials and articles intended to come in contact with foodstuffs and Repealing Directives 80/590/EEC and 89/109/EEC (L338/4), Brussels, 2004
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[3] J. Muncke, Exposure to endocrine disrupting compounds via the food chain: Is packaging a relevant source?, Science of the Total Environment 407 (2009) 4549–4559
[4] C. Nerin, P. Alfaro, M. Aznar, C. Domeno, The challenge of identifying non-intentionally added substances from food packaging materials: A review, Analytica Chimica Acta 775 (2013) 14–24

Keywords: *food contact materials, packaging contaminants, Non-Intentionally Added Substances (NIAS), GC/MS, odorous volatiles*

L108*
IMPACT OF COOKING ON FOOD CONTAMINANTS

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Food-producing animals can be exposed to various micro-pollutants during breeding. These micro-pollutants are rapidly transferred from the environment to animal edible tissues, thus representing a public health risk. Only a fraction of the micro-pollutants present in food is bioaccessible to the consumer due, for example, to technological processes applied to food before ingestion. Indeed, degradation by breakdown of thermolabile compounds or juice expelling of lipophilic or hydrophilic heat-resistant compounds can occur during cooking and induce a variation in the contaminant content of food. Therefore, worldwide food safety agencies encourage residue chemists to investigate the fate of toxicants during processes like cooking in order to upgrade their risk assessment procedures. The aim of the present paper was to study the impact of domestic cooking on the level of contaminants in food based on multiresidue methods. In a first step, a GC×GC–TOF/MS method w as developed to achieve a satisfactory separation of the 209 PCBs and the 17 toxic PCDD/Fs in neat solvent. The best GC×GC–TOF/MS conditions determined according to peak shape (width and symmetry) and resolution enabled to separate 206 dioxin-related micro-pollutants including the 17 PCDD/Fs. Starting with meat as a model matrix, the second step enabled to set up procedures for both micro-pollutant spiking and sample preparation. The later included accelerated solvent extraction (ASE), centrifugal evaporation and gel permeation chromatography (GPC). The performance of the ASE–GPC–GC×GC–TOF/MS method was assessed in terms of recoveries, reproducibility, linearity and LODs. In the third step, the multiresidue method was implemented to assess the modulating influence of cooking on meat content in the dioxin-related micro-pollutants. Finally, based on similar approaches and cooking procedures, this study was expanded to other meat contaminants such as heavy metals, pesticides an d antibiotics.

Keywords: *polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs), food spiking, multiresidue method, cooking*

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L109
NOVEL TEST APPROACH FOR EVALUATING BARRIER PROPERTIES OF FOOD CONTACT MATERIALS AGAINST MINERAL OIL CONTAMINANTS

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Recently, mineral oil (MO) residues in food have raised concern because there is a potential risk that consumers are exposed to a range of MO hydrocarbons (MOH) via food [1]. The European Food Safety Authority (EFSA) published an opinion on mineral oil in 2012 [1]. Concern was in particular expressed for the MO aromatic hydrocarbons (MOAH), because EFSA assumed the presence of genotoxic carcinogens. Potential background exposure to saturated MO hydrocarbons (MOSH) via food was also considered a concern. MOH contamination of food may be caused by printing ink residues found in packages from recycled paperboard but originates also from certain additives, processing aids, and lubricants. As a result of the concerns, an ordinance for food and food packaging is under preparation in Germany. The ordinance will set limits for MOSH and MOAH in food and food contact materials manufactured using recycled paper pulp, to protect consumers from potential health risks [2]. The crucial question is whether MOH compounds can penetrate through barriers at the food contact side of the recycled packages or indirectly through inner packages of virgin paperboard into into the food. Several methods to test the food contact materials for MOH transmission have been introduced [3,4] including the use of test packs with layers of donor paper, a spacer paper, the tested barrier layer and a receptor. An alternative method is the permeation cell method. A third alternative uses test cups for hexane vapour transmission rates measurements. The objective of this work is to develop a reproducible barrier test method where the mineral oils are introduced to the test system in the gas phase. The focus is on using surrogate compounds of a relevant molecular weight range for the drafted German ordinance. Finally, the equipment and materials used should be commercially available. In the new approach, the selected surrogate compounds representing MOH were introduced in the gas phase by saturating a closed space with them. Inside the closed space a fixed amount of food simulant, typically modified polyphenylene oxide Tenax™, was placed into commercial test cups. The materials tested for their barrier properties were fitted into the lid of the test cups. After the test period, the cups were removed from the closed space and the food simulant was extracted. Finally, the content of the surrogate compounds in the extract was determined by gas chromatography and the transmission rates were calculated. Boards with different barrier coatings were tested with this new method and theoretical diffusion constants were determined using a physical model for the experimental setup.

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[3] Grob, J. Verbr. Lebensm. (2014) 9:213–219
[4] Seyffer et al, BASF Test method

Keywords: *food contact materials, contaminants, mineral oil, barrier testing, physical model*

Acknowledgement: *Partial founding from BASF, Cargill, Huhtamäki, Kemira, Metsä Board, Stora Enso is acknowledged.*

L110
BISPHENOL F IS FORMED DURING THE PRODUCTION OF MILD MUSTARD

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Bisphenol F (BPF) was found in mustard up to a concentration of around 8 mg/kg. A contamination of the raw products or the packaging could be ruled out as potential sources of BPF. Also the fact, that only 4,4' BPF was found, spoke against a contamination from epoxy resin made from technical BPF or from bisphenol F diglycidyl ether, respectively. Only mild mustard made of the seeds of *Sinapis alba* contained BPF. In all probability BPF is a reaction product from the breakdown of the glucosinolate glucosinalbin with 4-hydroxybenzyl alcohol as an important intermediate. A formation mechanism is proposed and discussed. Hot mustard which was made only from brown mustard seeds (*Brassica juncea*) or black mustard seeds (*Brassica nigra*) contained no BPF. BPF is structurally closely related to the controversially discussed bisphenol A (BPA). BPF has weak estrogenic activity similar to that of BPA. The consumption of a normal portion of 20 g of mustard, for instance ea ten with a sausage, can lead to an intake of 100 to 200 µg of BPF. According to a preliminary assessment, the risk of BPF in mustard for the health of consumers is considered as low, but available toxicological data are insufficient for a conclusive evaluation. Other possible natural sources of BPF in foodstuffs are discussed. It is a new and surprising finding, that BPF is a natural food ingredient and that this is the main uptake route. This insight sheds new light on the risk linked to the family of bisphenols.

Keywords: *bisphenol F, mustard, Sinapis alba, glucosinalbin, 4-hydroxybenzyl acohol*

L111*
CLOSER LOOKING TO MINERAL OIL HYDROCARBONS (MOSH/MOAH) IN PAPER PACKAGING USED FOR FOOD CONTACT

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A currently heavy discussed theme in the field of food- and hygiene packages, produced of paper and board, concerns saturated and aromatic mineral oil hydrocarbons (MOSH – mineral oil saturated hydrocarbons and MOAH – mineral oil aromatic hydrocarbons). MOSH consist of linear and branched alkanes, and alkyl substituted cycloalkanes, whilst MOAH include mainly alkyl-substituted polyaromatic hydrocarbons up to four aromatic rings. The main focus is thereby on the aromatic fraction, which covers about 15–30% of the whole mineral oil fraction. The main sources of mineral oils in paper and board are offset printing inks either directly applicated to the package or introduced via the recycling process, as mineral oils are not completely removed during the recycling process. The transfer of mineral oil hydrocarbons from the packaging to the food can occur either by direct contact or through the gas phase, by evaporation from the packaging and recondensation in the packed good, whereupon relevant migration reaches up to hydrocarbons of n-C24 and noticeable migration reaches up to hydrocarbons of n-C28. Based on the acceptable daily intake (ADI) of 0.01 mg/kg body weight, specified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002 [1], a specific migration limit (SML) of 0.6 mg/kg of MOSH and 0.15 mg/kg of MOAH was derived using standard assumptions for calculation of the SML [2]. Most data on mineral oil in food and food packaging material were generated by on-line or off-line coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC–GC–FID) [3,4], determining the total concentration of MOSH and MOAH. Because of FID detection, which is necessary for the quantification of MOSH and MOAH, as it is the only detection method which provides the same response per mass of hydrocarbons, there is a lack of knowledge concerning the composition of the fractions. Besides these, it is not possible to resolve the fractions into individual components for identification, as they form broad humps of unresolved compounds. However using methods based on multidimensional gas chromatography, it is possible to distinguish and identify certain MOAH sub-classes, whereas comprehensive GC×GC appears to be the most effective method. This work focuses on the MOAH fraction using different comprehensive GC×GC set-ups (columns of different polarity, variation in the modulation frequency,…) for the identification of individual components, as well as different substance-classes of the MOAH. Furthermore the impact on the separation performance and efficiency of the different set-ups was considered.

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Keywords: *MOSH/MOAH, food packaging, paper, board, comprehensive GC×GC*

L112*
DETERMINATION OF MIGRATING COMPOUNDS FROM PLASTIC BABY BOTTLES BY GC–QQQ–MS AND LC–QQQ–MS

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After the European Union prohibited the production of Bisphenol-A-containing polycarbonate baby bottles (EU 10/2011), alternative materials, such as e.g. polypropylene, polyethersulphone, etc. have appeared on the Belgian market. The first aim of this study was the identification of major organic compounds migrating from these new baby bottles. After sterilisation by boiling during 10 min, 3 consecutive migrations with simulant D1 (water:EtOH (50:50) were performed at 70°C during 2h (EU 10/2011). Afterwards, the migration solutions were analysed using a liquid-liquid extraction with EtOAc:*n*-hexane (1:1). Various compounds, such as alkanes, phthalates, antioxidants, etc. were identified by GC–MS using commercial mass spectral libraries. Unidentified peaks were further investigated by GC–(EI)–TOFMS and GC–(APCI)–QTOFMS for structural identification. Additionally, extracts were analysed by LC-QTOF–MS to determine the less volatile migrants, such as Bisphenol-S. Based on these results, the most toxic migrating compounds were monitored and quantified using validated GC– and LC–QqQ–MS methods. An evaluation of the effect of several "real-life use conditions" such as microwave, sterilisation and dishwasher on the profile of the different migrants was determined and compared with the standard EU "repetitive use conditions" (3 migrations, 2h at 70°C). Analysis of the 3rd migration step of the standard conditions (which has to comply with the EU legislative migration limits) showed that for some baby bottles several not authorised compounds were observed exceeding the established "no-detection limit" of 10 µg/kg. Substances such as 2,4-di-*tert*-butylphenol (up to 118 µg/kg), 2-butoxyethyl acetate (up to 945 µg/kg) and 4-propylbenzaldehyde (up to 27 µg/kg) were detected in several bottles as well as some phthalates. The silicone bottle even exhibited concentrations of TXIB around 350 µg/kg, whereas this compound is only authorised for use in single-use gloves. For all detected compounds authorised by the EU 10/2011 with a specific migration limit (SML) such as benzophenone (600 µg/kg, found up to 97 µg/kg), concentrations were below the SMLs. Analysis of the extracts from the microwave experiments showed similar trends, but lower concentrations than those observed in the repetitive use experiments. Furthermore, a downwards tendency of these concentrations towards the subsequent microwave cycli was perceived. 4-propylbenzaldehyde was detected in one specific polypropylene bottle at max. 9 µg/kg after the first microwave treatment, whereas its concentration was 3 times higher in the 3rd step of the repetitive experiment. Other parameters, such as the influence of the sterilisation or the dishwasher on the concentrations of these migrants are still ongoing, yet these first results suggest that the conventional migration experiment prescribed by the EU overestimates the actual concentrations of compounds migrating from plastic baby bottles.

Keywords: *baby bottles, plastics migration, BPA alternatives, GC–QQQ–MS, LC–QQQ–MS*

L113
LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC–MS/MS) DETECTION OF GLYCIDYL ESTERS AND MCPD ESTERS IN INFANT FORMULA

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Fatty acid esters of 3-chloro-1,2-propanediol (3-MCPD), 2-chloro-1,3-propane diol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. Formed during the deodorization of oils during refining, these compounds are considered potentially carcinogenic and/or genotoxic, making their presence in edible oils and processed foods containing these oils a potential health risk. Recently, there has been increasing attention to the use of these refined oils in commercial infant formulas. Since formula is typically an infant's sole source of nutrition, and due to infant's low body weights, the presence of MCPD and glycidyl esters poses a potential health risk. At present, published validated methods for extracting and quantifying these contaminants in processed foods are limited to mayonnaise and salad dressings. The current work focuses on developing methodology for extracting and quantifying these chemica l contaminants from commercial infant formulas in order to determine levels of exposure. A method for extracting MCPD and glycidyl esters from infant formula will be described in this presentation. The extraction efficiencies of MCPD monoesters, MCPD diesters and glycidyl esters in a homemade infant formula with known MCPD/glycidyl ester concentrations will also be discussed. Results indicate that extraction efficiencies of greater than 90% can be achieved for all monoester, diester, and glycidyl ester species using the developed extraction procedure. Quantitation of MCPD and glycidyl esters was performed using a liquid chromatography tandem mass spectrometry (LC–MS/MS) method that was previously developed for the quantitation of these species in edible oils. Validation results indicate the extraction and quantitation methods developed for infant formula are sensitive and robust. Using the validated methodology, a survey of a number of commercially available infant formulas (in both the United States and abroad) was conducted in order to assess infant exposure to MCPD and glycidyl esters.

Keywords: *processing contaminants, MCPD esters, glycidyl esters, LC-MS/MS, infant formula*

L114
INTEGRATING LC–MS AND NMR FOR STUDYING BIOACTIVES' BIOCHEMISTRY

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Plants and foods are natural sources of bioactive compounds. These are often secondary metabolites of a wide variety of physic-chemical properties, able to induce effects on metabolism, by activating or inhibiting protein targets. Due to their chemical complexity, bioactives require state-of-the-art techniques to directly study them. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) are ideal partners for all small molecules applications, as they provide complementary information enabling the identification of bioactives from mixtures. Our group develops a variety of preparative and analytical strategies, including solid phase extraction (SPE), different chromatographies such as supercritical fluid chromatography (SFC) and liquid chromatography (LC), offline and/or online with MS and NMR to fractionate, isolate, identify, monitor, and quantify bioactives and its metabolites in mixtures. In particular for bioactive identification, we make use of an integrated LC–UV/Vis–SPE–NMR/MS system. This set-up, alone, provides a multitude of chemical information (retention time, UV/Vis, MS and fragmentation pattern, 1D and 2D NMR spectra) and combined with spectral databases and computational algorithms leads to the full structure elucidation in the most efficient way.

Keywords: *bioactives, NMR, MS*

L115
DEVELOPMENT OF NEW 19F NMR METHOD FOR THE CLASSIFICATION OF FOOD: APPLICATION ON THE AUTHENTICITY OF EXTRA VIRGIN OLIVE OILS

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The natural phenolic antioxidants, containing a function group –OH, are widely distributed in natural foods, such as olive oil, and have been found out to exhibit positive health effects in the prevention of various diseases associated with oxidative stress such as cardiovascular and neurodegenerative diseases and cancer. The molecules containing a -OH (OHs) are directly associated with the olive variety, geographical origin as well as adulteration and fruit ripening and processing. We like to introduce a new ^{19}F NMR method based on the fluoro-labelling of -OH chemical group of the compounds by suitable fluorine reagent for the quantitative determination of OHs in edible oils. The 100% natural abundance of the ^{19}F nucleus, the high gyromagnetic ratio and the small relaxation times (0.5–2 s) make ^{19}F NMR sensitivity nearly the same as that of a proton. Its chemical shift extends over a wide range providing adequate signal dispersion that reduce signal overlap and aid interpretation. The ^{19}F chemical shift data for the trifluoroacetate derivatives of OHs cover a range of ~2 ppm, exhibiting the following order, from low to high field: phenols > aliphatic polyalcohols > benzyl alcohols > primary alcohols > 6-membered cyclic secondary alchohols > secondary alcohols > aromatic acids > aliphatic acids > tertiary alcohols. Olive oil samples from different cultivars such as koroneiki and ladoelia, from Cyprus and Greece, were collected over a two years harvest and measured by the ^{19}F NMR method. Statistical analysis of the data was performed by Principal Component Analysis (PCA) and showed that the samples are classified based on the cultivars and on their geographical origin.

Keywords: *^{19}F NMR, EVOO, classification, phenolics, statistics*

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L116
MOLECULAR CHARACTERIZATION OF PECTIC OLIGOSACCHARIDES DERIVED FROM AGROINDUSTRIAL BY-PRODUCTS

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Pectin is a heterogeneous carbohydrate found in the cell wall of higher plants. It includes a family of acidic polymers, known as homogalacturonan and rhamnogalacturonan, with several neutral sugars/polymers such as arabinans, galactans and arabinogalactans attached as side chains. In the form of pectic oligosaccharides (POS), these polymers have been proposed as a new class of feed prebiotics able to exert several health promoting effects. They are not digestible by animal enzymes and induce changes in the composition and activities of the gastrointestinal microbiota. The extraction of these polymers in the form of POS is a promising step towards the manufacture of prebiotics from agricultural by-products. The aim of this work was to characterize the complex mixtures of POS obtained from food wastes, more specifically from sugar beet pulp and onion hulls, after extraction (by chemical or enzymatical means) and further enzymatical hydrolysis of pectins. To achieve tailoring of the POS, the enzymatic hydrolysis was performed in a cross flow continuous enzyme membrane reactor. Hydrophilic interaction chromatography (HILIC) coupled with electrospray mass spectrometry detection (ESI-MS) was used to identify POS on the basis of retention time features, molecular weight and in source fragmentation. These results were complemented by monomer and oligomer analysis using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD). The analyses provided important structural information, such as degree of polymerization, sugar composition and the presence of methyl esters and acetyl groups. The separation and (semi-)quantification of oligosaccharides present in the complex mixtures with different degrees of polymerization was obtained. The POS fractions from sugar beet pulp were also tested for their prebiotic activity; the bacterial growth of seven Lactobacillus strains and two E.coli strains, the latter chosen as being common pathogens for poultry and pigs, was monitored using the impedometric technique by mean of BacTrac® 4300 system. The in vitro studies show that some POS preparations show the ability to stimulate the growth of Lactobacillus strains and therefore have the potential to act as prebiotic in feed.

Keywords: *pectic oligosaccharides, agroindustrial by-products, hydrophilic interaction chromatography, MS, prebiotic test*

L117
EXPLORING BIOACTIVE PEPTIDES FROM MARINE SOURCES

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L118
OLIVE BIOACTIVES: POSSIBILITIES AND APPLICATIONS

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The olive tree, closely connected to the Mediterranean region has provided a wealth of goods. Research on the olive has started early but it has proven inexhaustible revealing mainly a vast array of nutritional and health properties. Apart from olive oil and table olives, the by-products coming from olive processing industry have been proven attractive materials for research. Over the years a multidisciplinary team has been established in NKUA with strong expertise in all aspects of olive investigation and exploitation. The aim of this communication is to present our holistic research strategy towards the multifaceted exploitation of the olive tree including activities such as extraction, fractionation, isolation, analysis of olive tree products as well as investigation of processes related to olive industry and valorization of by-products. The main products of the olive tree, olive oil and table olives as well as by-products such as leaves, paste, mill waste, table olive wastewater have been used as sources for the recovery of valuable secondary metabolites. This has been performed with conventional techniques and also by adsorptive resin technology. In addition standardized enriched fractions have been prepared with various techniques, such as MPLC, HPLC, and FCPC. Isolation of promising lead compounds with emphasis to olive biophenols oleuropein (leaves), hydroxytyrosol & tyrosol (olive oil, by-products), oleacein & oleocanthal (olive oil) and lactones (by-products), has been achieved. From this effort a unique library of extracts (approx. 50), enriched fractions (approx. 60) and compounds (approx. 55) has been created. Also, lab-scale processes have been adapted to pilot-scale systems for the isolation of extracts, fractions and compounds in high yield. Additionally, analytical techniques and meth odologies (UPLC/HPLC–DAD, HPLC–DAD–HR/MS^n, and HPTLC) have been developed and applied for the qualitative and quantitative determination of secondary metabolites in all the above mentioned materials. Furthermore, mapping of Extra Virgin Olive Oils (EVOO) from Greece is attempted the last 2 years. Towards this direction, NMR and ESI(±)-FT/ICR-based methods were employed for the analysis of the samples and the derived data were analysed using multivariate data analysis (MVA). The investigation of the biological profile and the therapeutic potential of olive extracts and compounds is also one of our goals and supported by several in vitro and in vivo studies while their possible application as nutraceuticals, dietary supplements, cosmeceuticals and cosmetics is also explored. Our efforts continue to expand in the direction of an integrated and holistic approach of research and exploitation.

Keywords: *olive oil, biophenols, mapping, analysis, isolation*

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L119
NOVEL FOODS ENRICHED WITH PHYTOSTERYL/-STANYL FATTY ACID ESTERS – NEW ANALYTICAL APPROACHES FOR A COMPREHENSIVE ANALYSIS

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A daily dietary intake of 2 g phytosterols/-stanols results in a reduction of LDL- and total plasma cholesterol of approximately 10%. Therefore, phytosterols and their fatty acid esters were among the first functional ingredients used to enrich foods in order to obtain an additional beneficial health effect. Their use in foods falls within the scope of the Regulation (EC) No. 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. The EU Commission decisions on authorizations include specifications of the profiles of both, the sterols/stanols and the esterified fatty acids used for enrichment. Owing to their structural resemblance, phytosterols are susceptible to oxidation reactions similar to those known for cholesterol. Comparable to cholesterol oxidation products, several in vitro studies showed cytotoxic and pro-inflammatory effects of phytosterol oxidation products (POPs). The number and the variety of foods enriched with phytosterols/-stanols and their esters are increasing. However, data on the contents of POPs are limited. Therefore, analytical methods were developed enabling (i) the analysis of individual phytosteryl and phytostanyl fatty acid esters in complex mixtures added to enrich foods and (ii) the determination of oxidation products that may be generated thereof. Establishing a UHPLC–APCI–MS-based method, a detailed qualitative and quantitative determination of the ester composition of e.g. enriched margarines could be performed. This is the essential basis for authenticity assessments as required by the aforementioned authorizations. In addition, an on-line LC–GC-based methodology was used as novel analytical tool for the analysis of POPs. Combining both analytical approaches, household ways of use and preparation of enriched foods, e.g. storage and electric heating of enriched margarines, could be characterized via the monitoring of qualitative and quantitative changes of individual intact esters and the concurrent formation of POPs. In conclusion, the established approaches allow a comprehensive characterization of novel foods such as phytosteryl/-stanyl fatty acid ester enriched margarines regarding product authenticity, quality and safety.

Keywords: *phytosteryl/ -stanyl fatty acid esters, phytosterol oxidation products, novel foods, on-line LC–GC, UHPLC–APCI–MS*

L120
TARGETED PROTEOMICS AS A NOVEL TOOL FOR THE AUTHENTICATION OF MEAT SPECIES

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Food adulteration is a of meat products is a problem of significant economic impact and routinely used methods such as ELISA and PCR can suffer from limited specificity or sensitivity especially in processed foods. Here we present the development of a mass spectrometrical multi-method for the sensitive and specific detection of up to nine different mammalian species in processed food using MRM and MRM3- based detection of species-specific tryptic biomarker peptides. These biomarkers were identified by a shotgun proteomic approach using tryptic digests of protein extracts from the nine different mammalian species included in our study. For each species about 5,000 peptides from approximately 500 proteins were identified and specificity of potential biomarker peptides was thoroughly assessed experimentally. The limit of detection for mammalian species is currently 0.05% in a beef matrix system. In addition, we were able to establish a rapid 2 min extraction protocol for the efficient protein extraction from processed food using high molar urea and thiourea buffers. Notably, prefractionation using 2D PAGE or OFF-Gel fractionation is not necessary and the method is therefore easily applicable in analytical routine laboratories without dedicated proteomics background.

Keywords: *food proteomics, mass spectrometry, biomarker peptides, authenticity*

L121
POTENTIAL OF MASS SPECTROMETRY METABOLOMICS IN CHEMICAL RISK ANALYSIS

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Food safety has become a major issue worldwide and in particular, detecting the presence of toxins, contaminants or residues of chemical substances along the food chain and in fine in food items constitutes a strong consumers demand. Generally all these substances and corresponding metabolites of interest are analysed using efficient targeted methodologies. However, in some cases these targeted approaches do not allow the detection of either those substances or emerging compounds/practices and therefore new approaches and strategies are demanded. Thereby the study of physiological perturbations induced upon exposure to a given chemical substance has emerged as an interesting alternative approach to be applied in chemical food safety [1,2,3]. This presentation is focus to describe the most significant applications of mass spectrometry based metabolomics in the field of chemical food safety. Through various examples, the different risk analysis steps (i.e. assessment, management and communication) will be addressed to illustrate such an approach is fit-for purpose answering the expectations and requirements of chemical risk analysis. It can be considered as an innovative tool to predict the likely occurrence and nature of risks, together with improving detection methods, in the aim of answering global food safety issues and anticipating human health problems.

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Keywords: *chemical food safety, risk assessment, risk management, risk communication, metabolomics*

L122*
USE OF VOLATILE COMPOUNDS IN LIVER AND PLASMA AS MARKERS OF ANIMAL EXPOSURE TO MICROPOLLUTANTS

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Diet is the major route of human exposure to a wide range of contaminants including environmental micropollutants, plant treatments or veterinary substances. To ensure consumer safety, detection and quantification techniques have to be set up for the determination of toxic contaminants in food, in particular in meat. At present, the methods used are generally complicated to set up and costly due to the high level of performance required. Their use is thus inconceivable for frequent and large-scale controls required to guarantee effectively the chemical quality of food to the consumer. In order to address this issue, the present paper introduces an alternative approach based on targeted quantification of livestock's exposure markers to pollutants. Among these compounds assumed as potential markers, the volatile organic compounds (VOCs) were identified as particularly promising biomarkers to detect several types of pathologies. The analysis of these volatile m arkers could enable to reveal systematically suspect samples on market and to guide further analyses to confirm and possibly explain the contamination. Based on animal tests involving laying hens contaminated or not by different contaminants (environmental micropollutants, pesticides, mycotoxins and veterinary substances) through their feed, the present study demonstrates the relevance of determining VOCs in liver and plasma by Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry (SPME–GC–MS) in order to reveal animal exposure to several classes of micropollutants. Firstly, volatile markers were investigated in hepatic tissues. The experiment consisted in adapting the SPME parameters to guarantee the best representativeness, the minimal analytical variability and the maximal sensitivity for the extraction of VOCs in animal liver. Candidate volatile markers of exposure to eight micropollutants including two environmental contaminants (PCBs, cadmium), two pesticides (lindane, deltametrine), two veterinary substances (ampicillin, monensin) and two mycotoxins (fumunosin and zearalenone) were then investigated. Metabolic signatures generated by SPME–GC–MS confirmed the discrimination obtained by SPME–MS signatures between contaminated and non-contaminated groups and enabled the identification of robust markers responsible for the discrimination. Secondly, volatile markers were determined in plasma to open the prospect of applying noninvasive analyses to back-trace contamination. SPME–GC–MS signatures were collected according to the strategy developed for the analysis of the VOCs in liver. The results demonstrate discrimination between control group and some contaminated groups. The correlation of the identified markers in plasma and liver has been studied to provide robustness to the volatile markers.

Keywords: *food safety, toxic contaminants, volatile biomarkers*

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L123
POTENTIAL OF THE REVERSED-INJECT DIFFERENTIAL FLOW MODULATOR FOR COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY IN THE PROFILING AND FINGERPRINTING OF VOLATILES FROM COMPLEX FOOD SAMPLES

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Comprehensive two-dimensional gas chromatography (GC×GC) coupled with Mass Spectrometry (MS) is one of the most powerful analytical platforms now available for the detailed profiling (identification and quantitation) of medium-to-high complexity food samples [1]. Compared to one-dimensional systems, it offers remarkable separation power and unmatched peak capacity, rationalized 2D separation patterns that are distinctive sample fingerprints for classification and authentication [2-5]. Thermal modulated GC×GC platforms enable comprehensive investigations required in food “omics” thanks also to their hyphenation with mass spectrometric detection, automated sample-preparation, olfactory detection and suitable data elaboration approaches. The introduction on the market of GC×GC platforms implementing differential flow-modulation, based on the design proposed by Seeley et al. [6], has opened a new perspective for complex samples analysis (profiling and fingerprinting). Lower operational costs, relative ease of use and simple maintenance make these platforms attractive also for routine operations. This study investigates and critically discusses the potential of a “second generation” design of differential flow modulation that adopts a reverse fill/flush injection dynamic for profiling and fingerprinting of medium-to-highly complex fractions of volatiles from different food samples. In particular, a parallel dual-secondary column dual-detection configuration was tested; this system has shown to improve the information potential also with thermally modulated GC×GC (MS identification reliability and accurate FID quantitation). This contribution presents chromatographic performance parameters and the most effective systems for quantitative profiling of flavoring agents (*Mentha spp.* essential oils) and fingerprinting of complex fractions from roasted samples (hazelnuts and cocoa). Experimental results show that a careful optimization of both column dimensions and system configurations yields accurate and reliable results compatible with routine quality controls and high throughput screenings.

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Keywords: *two-dimensional comprehensive gas chromatography-mass spectrometry and flame ionization detection, reverse-inject differential flow modulation, quantitative profiling, fingerprinting, chemical fingerprint*

L124*
UNTARGETED MASS SPECTROMETRIC ANALYSIS OF HEATED MILK FOR THE IDENTIFICATION OF NON-ENZYMATIC POST-TRANSLATIONAL B-LACTOGLOBULIN MODIFICATIONS

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Milk is usually heated to ensure microbial safety for consumers. However, the processing leads to numerous chemical modifications of milk proteins, e.g. by oxidation, glycation and glycoxidation of amino acids. The comprehensive knowledge of these modifications is essential for the evaluation of the physiological, toxicological, nutritional and technological consequences of thermal milk treatment. Therefore it is important to determine the structures of newly formed products as completely as possible. Many studies in this research field used a selective targeted approach to investigate thermally induced milk protein modifications, like lactulosyllysine and carboxy-methyllysine. However this strategy does not allow the detection of unknown modifications. Therefore it was our aim to develop an untargeted method, in which bioinformatic and mass spectrometric analysis were combined to elucidate post-translational protein modifications that may have escaped from identification so far. Two sample groups- the treated sample (ß-lactoglobulin heated with lactose for 3 d/7 d, set 1) and a negative control (ß-lactoglobulin, set 2) were used for this purpose. After specific enzymatic protein hydrolysis, the resulting peptides were analyzed by UHPLC-ESI–MS/MS in full scan mode. The acquired LC–MS–data were processed with the bioinformatic software XCMS which incorporates novel nonlinear retention time alignment, matched filtration, peak detection and peak matching to identify signals with statistically significant differences between the two data sets. The resulting XCMS list of the signals (*m/z* values, retention times, p-values, areas) was reduced by different applied criteria to focus on relevant hits (e.g. p-value, signal to noise ratio). In the next step enhanced-resolution- and product-ion-scans of the selected signals were performed to identify the structures of these modifications and to localize their binding sites either manually or by de-novo-sequencing. With this approach, well known modifications could be detected like lactulosyllysine, cysteine sulfonic acid, N-formylkynurenine or methionine sulfoxide. Additionally, we observed a mass shift of +28.0 Da at lysine, which could be assigned to N-formyllysine, which had not been detected in heated milk so far. Furthermore mass shifts of +12.0 Da at the N-terminus, -2.0 Da at tryptophan and +84.0 Da at arginine could be detected that have not been reported yet. In the next step a sensitive MRM method was applied to detect all modifications in milk and to monitor their site-specific reaction kinetics during the thermal treatment of milk. With this untargeted approach, which combines bioinformatic data analysis with mass spectrometric analysis, we were able to get a comprehensive overview of ß-lactoglobulin modifications in heated milk and to detect novel modifications that will be further analyzed for structure elucidation.

Keywords: *milk, ß-lactoglobulin, protein modification, mass spectrometry, untargeted approach*

L125
MOLECULARIZATION OF QUALITY CHANGES OF CARROTS (DAUCUS CAROTA *L.*) INDUCED BY ABIOTIC STRESS

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All over the world fresh carrots (*Daucus carota L.*) as well as its processed products are highly appreciated by the consumer for its typical pleasant sweet taste profile. Unfortunately, the attractive sensory quality of carrots is hindered by a sporadic bitter off-taste which is often the reason for consumer reactions and therefore a major problem for vegetable processors. This off-flavour is induced by abiotic and biotic stress factors during harvesting, transportation, storage and processing. Although recent application of a SENSOMICS approach gave first insight into individual bitter tasting secondary metabolites in carrots, their up-regulation during abiotic stress still remains elusive. Aimed at increasing our knowledge on the chemical mechanisms involved in quality changes of cultivated carrots in response to abiotic stress factors like water stress or post-harvest mechanical (transport-) stress, different stressed and native carrot genotypes were comparatively screened by application of a fast and robust high-throughput ultra-performance liquid chromatography/time-of-flight mass spectrometry (UPLC–TOF–MS) METABOLIC profiling analysis. Software-assisted selection of marker metabolites in stressed carrots, followed by preparative chromatographic purification revealed the chemical structures of several bitter stimuli by means of LC–MS, LC–MS/MS, and $^{1}D/^{2}D$–NMR experiments. The concentrations of these bitter compounds in carrots before and after stress challenge of the same genotypes could also be confirmed by means of targeted UPLC–MS/MS (MRM) analysis. These results might help to navigate carrot breeding programs and to optimize post-harvest treatment of carrots from producer to consumer/processor towards the production of sensorially preferred carrot products.

Keywords: *carrots, metabolomics, sensomics, abiotic stress*

L126
FLAVOROMICS APPROACH IN MONITORING CHANGES IN VOLATILE COMPOUNDS OF VIRGIN RAPESEED OIL CAUSED BY SEED ROASTING

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Two varieties of rapeseed (one high oleic, containing 76% of oleic acid, and the other – containing 62% of oleic acid) were used to produce virgin (pressed) oil. The rapeseeds were roasted at different temperature/time combinations (at 140–180°C, and for 5–15 min.); subsequently, oil was pressed the from roasted seeds. The roasting improved the flavour and contributed to a substantial increase in the amount of a potent antioxidant – canolol. The changes in volatile compounds related to roasting conditions were monitored using comprehensive gas chromatography – mass spectrometry (GC×GC-ToFMS), and the key odorants for the non-roasted and roasted seeds oils were determined by gas chromatography – olfactometry (GC–O). The most important compounds determining the flavour of oils obtained from the roasted seeds were dimethyl sulfide, dimethyl trisulfide, 2,3-diethyl-5-methylpyrazine, 2,3-butanedione, octanal, 3-isopropyl-2-methoxypyrazine and p henylacetaldehyde. For the oils obtained from the non-roasted seeds, the dominant compounds were dimethylsulfide, hexanal and octanal. Based on GC×GC-ToFMS and principal component analysis (PCA) of the data, several compounds were identified that were associated with roasting at the highest temperatures regardless of the rapeseed variety: these were, among others, methyl ketones (2-hexanone, 2-heptanone, 2-octanone).

Keywords: *flavoromics, rapeseed virgin oil, GC×GC-ToFMS, volatile compounds*

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L127
CHROMATOGRAPHY, WINNING EVERY BATTLE BUT LOSING THE WAR

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Chromatography, either or not in combination with mass spectrometry, is by far the most widely used method in food analysis. The technique is mature, meaning it provides a reliable tool for food analysis as well as for numerous other fields relying on accurate compositional information. Unfortunately most non-chromatographers see the maturity of the technique as a motivation not to invest in further developments any more. Chromatography is not the only method for detailed compositional analysis. Sensor technology, quantitative NMR, spectroscopy and many other methods are available. These techniques are considered much more 'scientifically original', sexy and amenable to grant funding than chromatography. Yet in terms of progress chromatography has a much better track record than many of the other techniques. Large volume injection methods allow us to improve sensitivity at least 10 fold, UHPLC has resulted in a threefold reduction in analysis time, comprehensive chromatography gives us a 10 times higher peak capacity, high resolution MS allows us to cover hundreds of compounds in a single run, etc. We have won all these battles. But do we get the credits? Chromatography, like all analytical techniques, is an enabling technique. We have to work with those on the forefront of life sciences, physics, materials research and other high impact areas. Through them we should get our credits. Unfortunately they choose other analytical techniques like NMR, other forms of spectroscopy or sensors. The main reason is the long time-to-first-results of chromatography. Users want their ideas confirmed quickly. Urgent food safety issues require an answer now. We are too slow in that. Chromatographers have to become part of the teams to discover new areas in the forefront science fields, not be hired in afterwards after the failure of the other technique. For that we have to show people what we can do. And give them some form of preliminary result soon afterwards. Reducing the time-to-first-results is key for a successful future. This will require technical improvements, such as generic methods and techniques with a higher compound coverage and resolution, as well as changes in our mind set: Fit-for-purpose triumphs over best-in-class. A whole range of exciting challenges opens up for us if we manage to do so. Localised compositional analysis, time resolved analysis, taking the instrument to the sample, molecules in context (neighbours and interactions) to name just a few.

Keywords: *chromatography, threats, universal methods, fast response, speed*

L128*
APPLICATION OF EMERGING PORTABLE AND NON-DESTRUCTIVE VIBRATIONAL SPECTROSCOPIC TECHNIQUES FOR ON-SITE AUTHENTICATION OF FOOD PRODUCTS

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Food fraud is a significant and growing problem, driven by globalization, economic opportunity, and the low probability and severity of punishment. On-site identification of food products suspect to adulteration is complicated for food safety inspectors, because the (often) vast amount of products visually do not deviate from their authentic counterparts. Adding up, the official laboratory analysis performed subsequently is lengthy and costly, and therefore restricting the amount of samples which can be analysed. A nowadays classical way to pre-screen samples for fraud in a laboratory are vibrational spectroscopic techniques like near-infrared (NIR), Raman and UV-Vis. These techniques rapidly provide information on macro-component level, and can be linked to a chemical parameter for which legal limits are established. A disadvantage is that inhomogeneous samples (meat, bakery goods, fruits, vegetables, etc.) need to be homogenized prior to analysis, meaning both package and sample are destroyed. In order to circumvent on-site selection problems, increase laboratory success-rates and prevent unnecessary sample destruction, we investigated the application of miniaturized NIR, Raman an UV-Vis equipment for on-site and non-destructive authentication using a number of food matrices. As an example, the freezing history (fresh-thawed), the presence of foreign additives (salt and porcine gelatine) and the legal moisture – protein ratio of chicken fillets (65 pieces, approximately 325 spectra) were investigated by portable NIRs by acquiring spectra directly on the meat surface. Multi-variate data analysis showed that, due to the inhomogeneity of the samples, 'classical' principal component classification and regression methods show little correlation with the values obtained in a laboratory. To overcome this problem, self-learning algorithms were exploited. In this case, support vector machine (SVM) classification and regression gave accurate results. For classification of chicken fillets in the classes fresh, thawed or containing additives, false negatives (1%) and false positives (3%) were in an acceptable range after extensive external validation. Furthermore, for moisture-protein quantification, small deviations from the actual values obtained using the corresponding ISO methods were found (2%). Multi-variate analysis of other food fraud applications investigated by portable vibrational spectroscopy, for example distinguishing organic and conventional produce types of meats and edible oil authentication, showed similar trends in approach. Finally, preliminary results show that SVM classification and regression models were not obstructed by transparent packaging materials, like plastic foil and glass. These results could pave the way for real-life development of on-site food authentication screening tools for inspection personnel.

Keywords: *miniaturized portable spectroscopics, chemometrics, support vector machine, food fraud*

L129
ION MOBILITY STRATEGY TO UNMASK STEVIOL GLYCOSIDES COMPOSITION OF ADDED SWEETENER IN FOODS

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For over a century, leaves from the shrub Stevia rebaudiana have been used as a sweetener and only in 2011 extracts were permitted to be used as a food additive (E 960) in the European Union. At first, flavoured drinks and table top sweeteners were the largest market opportunity; nowadays E960 is used in many food categories such as dairy products, confectionery, sauces (ketchup) and deserts. The composition of commercial steviol glycosides sweetener available has to be minimum 75% of Stevioside and or Rebaudioside A and not less than 95% stevioside, rebaudiosides A- F, steviolbioside, rubusoside and dulcoside on the dried basis. From these requirements, E 960 can contain up to 5% of glycosides not comprised in the EU recognized list of 10. Hence, characterizing these compounds (known 95% and unknown 5%) in many complex food matrices is a thrilling challenge for the analyst. In addition, steviol glycosides are difficult to separate by conventional reverse p hase chromatography, they are often subject to in source fragmentation and present isomeric pairs (e.g. Stevioside/Rebaudioside B) with similar physical properties. In this context, data interpretation is very complex and reliable identification can be unreachable. Ion mobility (IM) is known to be a powerful analytical tool for the separation of complex samples and have been extensively used for characterization purposes. This presentation will demonstrate the use of ion mobility features in conjunction with liquid chromatography and high resolution mass spectrometry for comprehensive characterization of steviol glycosides in food matrices. To establish the analytical protocol the following steps were first performed; 1) UPLC–IM–MS generic parameters were tested with pure standards of steviol + 10 steviol glycosides, 2) measured collision cross sections (CCS), retention times and fragmentation patterns were inserted in a scientific library for automatic search. From these r esults, ion mobility has demonstrated to be a powerful selective tool to separate critical isomeric pairs with up to 10 Å square difference between their CCS values. In addition, when performing real sample analysis, other possible non-targeted glycosides were detected and based on respective CCS values they were identified separately from the 11 targeted compounds even when co-elution occurred.

Keywords: *steviol glycosides, food additive, ion mobility mass spectrometry, CCS*

L130
NONDESTRUCTIVE IMAGING AND QUANTITATIVE ANALYSIS OF FOOD MICROSTRUCTURES USING X-RAY MICROTOMOGRAPHY

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Knowledge of the nano-, micro and macrostructure of food products and its intricate relation to product properties is necessary to develop new and better products. Consumers need high quality food products with a superior taste, texture, freshness and shelf life that are ready-to-eat or quick and easy to prepare. These food products contain complex structural features such as pores, droplets, crystals, interfaces and networks. Making these structures visible and quantifying them is essential to develop products with optimal product properties. X-ray computed micro-tomography (µCT) is an essential part of a broad range of complementary imaging techniques used to visualise structure elements during processing and storage. Detailed information can be obtained about the 3D internal microstructure of food products, prototypes and materials in a non-destructive way. This allows further measurements and tests on the same sub sample using other imaging or analytic al techniques. Examples will be given of the application µCT and MRI and quantitative image analysis methods to obtain multi-length scale information of the porous structure of carrots in their freeze-dried, rehydrating and rehydrated form. This work established a predictive relation between freezing rate and freeze damage. Water imbibition rates could be explained from the porous structure induced during freeze-drying. The non-destructive nature of µCT allows the investigation of the stability of aerated food products by imaging exactly the same sub volume in time. Dairy based products, such as whipped cream, dessert toppings and mousses are manufactured in the form of an emulsion and subsequently aerated to a foam. Air bubbles in these products have to be stable in time between production and consumption. Especially the stability of liquid and soft solid foams is a critical issue. They are thermodynamically unstable resulting in an increase in bubble size (coarsening) in time and eventually to complete loss of air. Frozen ice cream is much more stable. However during storage, there's still as driving force of air bubble coarsening. The sizes of air bubbles and ice crystals is critical to the ice cream's quality and sensory attributes. Large crystals lead to a coarse, grainy and icy texture. Whereas large bubbles will decrease the smoothness and creaminess. The size of ice crystals and air bubbles have to remain below the threshold of perception. Synchrotron µCT can be used for the in-situ observation and analysis of bubbles and ice crystals during temperature cycling of ice cream.

Keywords: *microstructure, imaging, micro-CT, X-ray microscopy, non-invasive*

L131
ANALYTICAL CHALLENGES FOLLOWING RECENT AND FUTURE DEVELOPMENTS IN EU POLICY ON CONTAMINANTS IN FEED AND FOOD

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Directive 2002/32/EC of 7 May 2002 of the European Parliament and of the Council on undesirable substances in animal feed is the framework for the European Union action on undesirable substances in feed.
Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food is the framework for the Union action on contaminants in food.
Following requests of the European Commission, the Panel on Contaminants in the Food Chain (CONTAM) from the European Food Safety Authority (EFSA) has completed in recent years several scientific opinions on contaminants in feed and food, reviewing the possible risks for animal and human health due to the presence of these substances in feed and food.
The outcome of these risk assessments has resulted in several changes to the EU legislation on contaminants in feed and food, such as inorganic arsenic, ergot sclerotia and tropane alklaoids. An overview of the recent changes with information on the considerations resulting in these legal provisions and the analytical challenges following these recent developments will be provided in the presentation.
In recent years, an increased prevalence and a significant year-to-year variation of the presence of mycotoxins in feed and food in the European region can be observed. Climate change and extreme weather conditions are considered to be the main cause. The high levels of aflatoxin in the maize harvest 2012 and the high level of Fusarium toxins in the maize harvest 2013 and 2014 have resulted in problems for feed and food supply and safety.
This situation entails specific challenges for farmers, feed and food manufacturers, traders and regulators to ensure the safety for animal and human health of feed and food while ensuring the supply of major staple fed and food such as cereals. This issue was intensively discussed in recent years and the presentation shall provide more details on these discussions and explore some possible options on the way forward.
Focusing on food, recently a lot of attention has been paid from an EU regulatory point of view to the presence of plant toxins in food and this is likely to increase in the future: erucic acid, pyrrolizidine alkaloids, tropane alkaloids, hydrocyanic acid … More details will be provided in the presentation.
Also in the field of the metals interesting developments are ongoing as regards the presence of (methyl)mercury and nickel in food;
Finally not to forget the processing contaminants such as acrylamide, MCPD-esters and glycidyl esters and in the future furan in food.
All these regulatory developments entail important analytical challenges!
To conclude, this presentation will bring you fully up to date on what is ongoing and what can be expected in the near future as regards EU policy on contaminants in feed and food!

Keywords: *contaminants in feed and food, analytical challenges, recent and future developments in EU policy*

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ALLERGENS

(A1 – A12)

A1
EFFECT OF THERMAL TREATMENTS ON HAZELUNT AND PEANUT ALLERGENICITY

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Food allergy represents an important health concern especially in highly industrialized countries. Tree nuts, and in particular hazelnuts and peanuts, can trigger severe hypersensitive immunogenic responses in sensitive subjects. The prevalence of these allergies is steadily growing worldwide, although the reasons for this increase still remain unclear [1]. In the last years attention has been paid to find suitable approaches based on chemical and physical treatments to significantly reduce allergenicity in different food products [2]. On the other hand, thermal treatments might enhance the final allergenicity, as demonstrated for certain foods [3]. In other cases, food processing is able to promote disruption of IgE epitopes with consequent allergenicity reduction [4]. In the present study, we investigated the effect of physical processing methods on the immunogenic potential of hazelnut and peanut allergens. Raw hazelnuts and peanuts were autoclaved for different time ranges and at different temperatures. The resulting protein fractions were analysed by SDS-PAGE electrophoresis and allergens were determined by ELISA and western blotting experiments, using sera of allergic subjects as a source of specific IgE antibodies. Autoclaving samples for 20 min at 210 kPa and at the temperature of 134 °C significantly reduced the intensity of the major allergen bands. In vitro experiments carried out with serum of allergic patients confirmed a decrease of IgE immunoreactivity with protein extracts from hazelnuts and peanuts thermally treated compared to the control. In conclusion, the combined exposure to high temperature and high pressure could be intended as an effective strategy to reduce hazelnut and peanut allergenicity in sensitive individuals.

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Keywords: *nuts, peanuts and peanuts, food allergens, processing, allergenicity*

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A2
MULTI-ALLERGENS DETECTION BY FLOW CYTOMETRY IMMUNOAFFINITY

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The prevalence of food allergies raised sharply over the past two decades, requiring the introduction of strict legislation on the declaration of ingredients in ready meals. Appropriate labeling is effective to minimize the risk of contact for people sensitized with the offending food allergen, but it is useless in the event of cross-contamination, where the presence of allergen is coincidental. Control strategies put in place by industrials and the health authorities are mainly based on the detection by ELISA of allergenic proteins in food. Although sensitive and specific, this method enables the detection of a single allergen per analysis. Effective control policy involves to conduct multiple tests to cover all allergens. We present in this work the results obtained with a method of multi-allergen detection using flow cytometry immunoaffinity (FCIA). In the developed test, microbeads coated with allergen proteins compete for antibodies against allergenic contaminants present in the extracted food sample. The amount of antibodies bound to the microbeads is inversely proportional to the concentration of allergens in the sample. Each allergen targeted by the method is coated on a set of microbeads displaying distinct spectral characteristics, allowing for identification when passing in front of the cytometer detector. Starting from 0.5 g of sample extracted in a saline phosphate buffer (PBS, 50 mM, pH 7.5), the method is able to quantify five allergens simultaneously (milk, egg, peanut, mustard and crustaceans) in cookies. Limit of quantification (LOQ) range between 0.25 ppm and 1 ppm depending on the targeted allergen. Further testing indicated no discrepancies for sensitivity and specificity between singleplex and multiplex format. Overall, FCIA could become a useful tool for routine quality control procedures in food industry.

Keywords: *cytometry, allergens, detection, multiplex*

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A3
LC–MS/MS DETECTION OF PEANUT AND ALMOND ALLERGENS IN SPICES

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Recent findings (in February 2015) of allergens in spices caused the recall of many food products in North America and Europe. The US Food and Drug Administration (FDA) advised people who are highly allergic to peanuts to consider avoiding products that contain ground cumin or cumin powder, because some shipments of these products have tested positive for undeclared peanut protein. The Food Standards Agency (FSA) has issued a further allergy alert following confirmation that a batch of paprika was the most likely source of undeclared almond protein in three food products which had been recalled. According to the European Rapid Alert System for Food and Feed (RASFF) portal, additional food products containing Cayenne pepper and Pilli-Pilli powder were found to contain undeclared traces of peanuts. It is important that consumers know food is safe and authentic. Potential weaknesses in the food supply chain need to be identified and counter measures need to be taken to strengthen consumer protection. Accurate and reliable analytical methods are needed to monitor the food supply chain and to allow correct labeling of food products. Here we present a method to detect the presence of peanut and almond in spices. Samples were extracted and then proteins were reduced, alkylated and digested using trypsin. The extracts were filtered and analyzed by LC–MS/MS using a reverse phase chromatography and positive ESI. The SCIEX QTRAP® 4500 system used for this study was operated in Multiple reaction Monitoring (MRM) mode to achieve high selectivity of detection. At least 12 transitions (3 transitions for 4 peptides) were monitored per allergen to minimize potential false positive results caused by matrix interferences.

Keywords: *allergens, LC–MS/MS, peanut, almond, quantitation*

A4
ANALYSIS AND QUANTIFICATION OF PEANUT PROTEINS IN A COMMERCIALLY AVAILABLE ALLERGEN QUALITY CONTROL KIT (CHOCOLATE DESSERT MATRIX)

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The analysis and detection of allergens is of great interest to the food industry and the most common technique for the analysis of peanut allergens used today is based on ELISA technology. In recent years, there has been interest in the use of mass spectrometry to perform this type of analysis, as LC-MS methods are able to target specific marker peptides that relate to the allergenic proteins, and can also provide quantitative results. The aim of the present study was analyse and quantify peanut presence in a commercially available allergen quality controlsample in which peanut protein (as roasted peanut flour) has been added to a chocolate dessert at 10 ppm. The matrix is based on that used in the EuroPrevall study for low-dose threshold studies in food allergic individuals. The two techniques were used to determine peanut protein content in the chocolate dessert matrix. ELISA analysis was undertaken using the standard protocols (as recommended by the manufacturer's instructions) to determine peanut concentrations. For the LC–MS analysis, the extraction and digestion steps have been optimised for the detection of 7 major peanut allergens and a peptide discovery experiment was performed in order to determine the most appropriate MS-based peptide markers. The experimental information was then transferred to a tandem quadrupole MS system, and isotopically labeled peptides (and their MRM parameters) were included as part of the LC–MS method. This poster will show representative data from the study for both ELISA and MS techniques.

Keywords: *allergens, LC-MS, peanut, ELISA*

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A5
ALLERGEN DETECTION IN PROCESSED FOOD PRODUCTS BY UHPLC–MS/MS

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Currently, 4 to 8% of children and 1 to 2% adults suffer from food allergies worldwide. To allow the allergic consumers to protect themselves by avoiding food that contain allergens, a reliable and sensitive analytical method is essential for the accurate labelling of food products reporting types of allergens they contain. In recent years, several methods have been developed and are now available to detect and quantify allergens including the most largely used ELISA tests. However, due to the homology between some proteins from different origins or process-induced modifications, immuno-based methods lead frequently to false positive or false negative results. UHPLC–MS/MS (ultra high performance liquid chromatography coupled to tandem mass spectrometry) is gaining more and more interest to analyze peptides derived from allergens contained in food because of its specificity, its sensitivity and the possibility to develop multi-allergen detection methods. The goal of this work was to detect milk, egg, soy and peanut allergens contained in a complex and processed matrix by UHPLC–MS/MS in order to test different extraction methods allowing the best determination of marker peptides. Incurred cookies were selected as targeted matrix due to both its complex composition (flour, sugar, oil…) and the heating-processed material (baking at 180°C during 18 min). An open-source software Skyline was used for the selection of MRM (Multiple Reaction Monitoring) for egg, milk, soy and peanut peptides and methods were transferred to an Acquity UHPLC–Xevo TQS system (Waters). The complexity of the matrix and the thermic conditions during process require the selection of an efficient extraction protocol with conditions allowing the allergen extraction that are compatible with a low detection threshold/high sensitivity. Therefore, we first optimized the protein extraction conditions. We tested the effects of the buffer composition (TRIS-NaCl, TRIS-HCl, NH_4HCO_3 or PBS), different concentrations in urea (a chaotropic agent), different pH, several extraction periods at two different temperatures as well as the extraction volume/sample amount ratio on both the total protein content recovered in the different samples (BCA) and the abundance of peptides that could be detected after a tryptic digestion and UHPLC–MS/MS analyses. Among the different conditions that have been tested, the extraction of proteins at 60°C for 30 min (with 15 min of ultrasound) in 200 mM TRIS-HCl (pH: 9.2) and a ratio of 5 for buffer/sample volume gives the best results as assessed by statistical analyses. Indeed, using this protocol, without any further sample purification, a LOD (Limit of Detection) of 10 ppm for milk proteins, 50 ppm for egg proteins, 10 ppm for peanut proteins, or 25 ppm for soy proteins could be reached. In the future, to improve the LOD, the samples will be concentrated by purification and other matrices will be tested to develop a unique multi-allergen method.

Keywords: *food allergens, mass spectrometry, multi-allergen analysis, optimization*

A6
A QUICK TEST FOR THE DETECTION OF PROCESSED AND UNPROCESSED SOY – RIDA®QUICK SOYA (R7103)

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Soy belongs to the so called big 8 allergens, therefore it has to be labeled as a food ingredient, nearly worldwide. Already small amounts of soy proteins can induce allergic symptoms at a dose of more than 5 mg soy protein. Especially processed food may contain soy proteins undetected by common methods, due to the lack of adequate antibodies and efficient extraction. R-Biopharm developed a dip stick suitable for the detection of raw and strongly heated soy in food. The antibodies used in this assay show - among other soy proteins - a specific binding to heated glycinin (Gly m 6), one of the major allergens in soy and β-conglycinin (Gly m 5) another important allergen. A unique extraction at 100°C allows the denaturation of all soy proteins and an efficient detection by the antibody. This dip stick is suitable for the detection of soy in production line for hygiene purposes, product testing has to be validated in the future.

Keywords: *allergen, soy, dip stick, lateral flow, hygiene*

A7
SCOPE OF SAFE IRRADIATED FOOD FOR SECURITY FORCES AND VICTIMS OF DISASTERS IN RCA COUNTRIES

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The results of the R&D studies conducted over the past thirty years have shown that food irradiation as an alternative treatment in addressing food-related issues: safety, security, global competitiveness and market access. The food industry's interest in the process is emerging for many reasons like persistent high food losses from insect infestation, microbial contamination, spoilage, and concerns over food-borne diseases. Therefore, food irradiation is gaining acceptance worldwide and is considered the technology of future. In June 2015, a Meeting on "Potential benefits of Irradiated Foodsfor Relief Organizations and Security Forces" was held under IAEA TC Project RAS 5/061in Chengdu, People Republic of China. The main objectives of the meeting was to put in contact irradiation specialists with representatives of security forces, relief organizations and other target groups in Asia and Pacific region that could benefit from the food irradiation. The chemical, microbiological and technological aspects of irradiation were discussed in detail. Representatives from IAEA, and Thailand gave an overview of the use of irradiation for food across the world. Chinese delegate presented ready-to-eat Chinese food that were developed but have not yet been utilized for relief operations. Indonesian participant shared her experience supplying meals ready to eat (MREs) to victims of a landslide in Java in 2014 when 9,000 packs of 100 g each were distributed. In May 2015, similar products were shipped to victims of an earthquake in Nepal. Philippines expert presented the characteristics of an enriched cereal bar having shelf-life of six months. Food specialist from Pakistan presented different types of irradiated MREs designed for security forces included flat bread, various curries and biryani (flavoured rice with meat). These MREs are included in a set also containing an accessory with solid alcohol to heat the MREs and also the water for tea. Half a million packs of 1.3 kg are prepared each year in Nowshera and irradiated in Lahore. The characteristics of MREs adopted by Pak Army on commercial scale include light in weight, nutritious, easy to carry, as per local taste/flavor, long shelf-life (up to 1 year), no special storage conditions be required, soft packing, suitable size to fit combat gear and cost effective. Korean expert presented emergency food that is used in the International Space Station. The technology has now been transferred to a private food company and the commercialization is pending. Challenges faced during relief operations in Malaysia and Jordan ware presented.

Keywords: *irradiated foods, relief organizations, security forces, Asia and Pacific Region*

A8
THE OCCURRENCE OF ALLERGENS IN "ALLERGEN-FREE" LABELED PRODUCTS

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Many people have allergic reactions to food. Most food allergies cause non-severe reactions but some responses are severe or life-threatening. Since there is not a cure for food allergies the protocol is to avoid foods that contain the specific allergen. The United States and European Union have strict guidelines on labeling. The eight most common food allergens are: milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybeans. There are many more food allergens in existence these are just the most recognized. A limited study was performed by obtaining allergen-free labeled products purchased from several different retail stores in the US. Analysis of these products was performed using ELISA technology.

Keywords: *food allergens, survey, ELISA*

A9
THE DEVELOPMENT AND VALIDATION OF REVEAL® FOR MULTI-TREENUT, A SINGLE LATERAL FLOW DEVICE FOR THE RAPID DETECTION OF WALNUT, ALMOND, HAZELNUT, CASHEW, PECAN AND PISTACHIO IN CIP AND ENVIRONMENTAL SAMPLES

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A lateral flow device for the simultaneous detection of six tree nuts was developed to rapidly screen for Walnut, Almond, Hazelnut, Cashew, Pecan and Pistachio. This sandwich based assay utilises specific antibodies for capture and additional antibodies linked to coloured particles for detection. A common extraction process has been developed to allow for detection of all six tree nuts on a single device. This unique multi-analyte product has been validated for various sample types including clean in place (CIP) and environmental samples. Reveal for Multi-Treenut is an accurate, rapid and simple to use assay that requires no special tools or extensive training and yields results in only 11 minutes from sample collection. The lower limit of detection is 5–10 ppm of all six tree nuts in CIP solutions and 5–40 µg/100 cm^2 for the tree nuts on stainless steel, plastic and Teflon. Purpose: The purpose of this study was to develop and validate Reveal for Multi-Treenut for detection of Walnut, Almond, Hazelnut, Cashew, Pecan and Pistachio in CIP solutions and environmental samples and to determine the limit of detection of this assay.

Keywords: *lateral, flow, tree, nut, allergen*

Acknowledgement: *I would like to thank Frank Klein, Mohamed Abouzied and Dave Almy for their technical support as well as FARRP for their services and expertise. In addition I would also like to thank Stephen Holmes and James Herbert for their continued support throughout the project.*

A10
TRACING ALLERGENS IN ONE-SHOT APPROACH – A MULTIPLEX LUMINEX ASSAY FOR SIMULTANEOUS DETECTION OF ALLERGENIC SOY PROTEINS

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Food allergies represent a threat for a rising number of people and affects up to 5–7 % of the population. According to the uniform EU rules soy is one of 14 food allergens that have to be declared in the ingredients list of any food. Doubtless detection of traces of allergens, however, poses a challenge up to now. Thus, provision of detection procedures to determine allergenic soy food components for food industries is important to satisfy the given EU regulation and consumer's wishes. So far, enzyme-linked immunosorbent assay (ELISA) is the most used technology to detect allergenic soy components. Its easy to use and relatively favorable format makes it suitable in laboratory food and feed testing at low ppm range. One drawback of ELISA, however, is the monoplex detection format, because soybean protein comprises of more than one allergy inducing structure. In the last decade 21 allergenic proteins in soybean have been identified, which possess numerous allergenic epitope s. This includes the storage proteins glycinin (Gly m6) and β-conglycinin (Gly m5), the Kunitz soybean trypsin inhibitor (Gly m TI) and the pathogenesis-related protein PR-10 (Gly m4). Currently, most of commercially available soy test kits are based upon generic soy protein and polyclonal antibodies without a reflection about defined antigens and epitope structures. Our goal was to combine the detection of current known allergenic soy proteins in a one-shot bead-based immunoassay (Luminex technology) using well-characterized monoclonal antibodies with stable quality. The combination of the ELISA-like immunoassay principle and the multiplex one-shot approach provides the possibility to develop a high-throughput, cost and time efficient soy allergen test kit. The Luminex technology is a fluorescence-based microbead immunosorbent assay offering the opportunity to examine up to 500 analytes in one well. Monoclonal antibodies are linked covalently on 5.6 micr on-sized polystyrene microspheres, which are internally dyed with red and infrared fluorophores. The capture antibody on the magnetic microspheres traps the analyte and after a magnetic separation step all unbound proteins are washed off. The second analyte-specific detection antibody labeled with another fluorescence-based reporter system allows quantification of the analyte by extrapolation to a calibration curve. As each microsphere carries a unique signature, the xMAP detection system can identify to which set of capture antibodies it belongs. Here, we present the development of a novel multiplex assay for detection of soybean-derived antigenic proteins, based upon newly generated high-affinity monoclonal antibodies for detecting Gly m4, Gly m5 and Gly m TI, which can be expanded with other antigenic proteins of interest.

Keywords: *Soybean, Gly m4, Gly m5, Gly mTI, high-throughput allergen detection, Luminex xMAP*

Acknowledgement: *This study is funded by MAVO Project of the Fraunhofer-Gesellschaft.*

A11
DEVELOPMENT AND CHARACTERIZATION OF A 2ND GENERATION COMPETITIVE ELISA FOR QUANTITATIVE DETECTION OF GLIADINES IN FOOD

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Celiac disease (CD) is a gluten-sensitive disease that damages the small intestine and interferes with absorption of nutrients from food. People with CD cannot tolerate gluten, a common name for gliadines and glutenines, water insoluble proteins found in food processed from wheat and related grain species, particularly barley and rye. The Leiden University Medical Center (LUMC) has developed and characterized monoclonal antibodies against gluten-derived T cell immune-stimulatory epitopes (toxic epitopes), involved in CD. Based upon the anti-α20 gliadin antibody from this study and following the Gluten-TEC® ELISA, a sensitive competitive α20 Gliadin ELISA was developed that detects a well-characterized toxic epitope of gliadin (α20) and homologues sequences present in wheat, barley, rye and their crossbred varieties. In this α20 Gliadin ELISA a synthetic peptide is used for both calibration and conjugate, allowing an accurate and reproducible standardization. The principle of the α20 Gliadin ELISA is as follows. To the wells of the ELISA plate coated with anti-α20 antibody, either α20 gliadin standard peptide or sample is added, immediately followed by addition of α20 gliadin peptide-HRP conjugate. After 3 hours incubation at +4°C, the wells are washed after which the amount of bound conjugate is visualized by addition of peroxide/TMB substrate. After incubation of 30 min at room temperature, the color intensity is measured at 450 nm. The obtained signal is inversely proportional to the amount of gliadin present in the sample. Several sample preparation methods were investigated. The easiest method giving the best recovery was found to be an extraction method with 60% of ethanol in water. Several beer, soup and sauce samples were tested, demonstrating that the ELISA can also be used to measure hydrolyzed/processed gliadines. Specificity of the test was investigated with several cereals. Positive results (>4 ppm) were obtained with wheat, rye and barley. Negative results (<4 ppm) were obtained with oats, rice and maize. Sensitivity of the test was determined in oats. LOD and LOQ were 2.9 ppm and 3.6 ppm, respectively. Recoveries were determined in oats, spiked with 5 ppm and 10 ppm of the Prolamin Working Group (PWG)-standard. The mean recoveries for 5 ppm and 10 ppm were 108% and 96%, respectively, showing a good correlation with the PWG-standard. Furthermore, oats was spiked with 5 ppm and 10 ppm of wheat, rye, or barley. The mean recoveries for 5ppm wheat, rye or barley were 108%, 112% and 125%, respectively, whereas the mean recoveries for 10 ppm wheat, rye or barley were 111%, 97% and 111%, respectively.
Take home message:
This 2nd generation competitive EIA for the detection and quantification of both native and processed gluten is characterized by
1 An easy to perform protocol
2 A simple ethanol extraction procedure without other hazardous chemicals
3 A well defined synthetic peptide standard for the calibration and the enzyme conjugate
4 Results available within 3.5 hrs
5 A single conversion factor from peptide standard to gluten concentration
6 No cross reactivity with oats.

Keywords: *Celiac disease, glutenines, anti-α20 gliadin antibody, Gluten-TEC® ELISA, gliadines*

A12
AN INCURRED COOKIE MATERIAL WITH DEFINED AMOUNTS OF A MILK PROTEIN CONTAINING STANDARD REFERENCE MATERIAL FOR ALLERGEN DETERMINATION

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Standardization of methods to determine allergens is absolutely necessary in the near future to fulfil the requirements of the food industry and sensitized consumers. To relate the results of different test kits to each other and to possible threshold values in the future, an “analytical anchor” is needed. It was the aim of this study to not only produce such a material but also mimic a natural milk contamination in a heat treated product as realistic as possible. Therefore, wheat flour was thoroughly mixed with a MoniQA skim milk powder reference material with known protein content to different levels. Afterwards other ingredients mainly sugar, egg yolk and margarine were added to the contaminated wheat flours with different milk protein contents. Cookies were formed, baked, dried and grinded. Milk protein contents were 0, 5, 10, and 30 mg/kg. These materials were produced and used independently by a commercial laboratory in course of the AOAC Performance Tested Method℠ Program to check the RIDASCREEN®FAST Milk ELISA (R-Biopharm AG, R4652). Samples were blinded before analysis, extracted five times for each level and analysed. Later on, samples were also checked with test kits of different manufacturers to characterize the overall suitability of this kind of material.

Keywords: *food allergens, reference material, milk protein*

AUTHENTICITY, TRACEABILITY, FRAUD

(B1 – B64)

B1
PCR MELTING CURVE PROFILES AND PRINCIPAL COMPONENT ANALYSIS DETERMINE PINE SPECIES

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Introduction. A dramatic peak in pine nut related dysgeusia, also referred to as pine mouth or pine nut syndrome (PNS) was observed in Europe in 2009–2011. Evident from the rapid alert (RASFF) database, PNS still occurs. The symptoms of PNS are characterized by a constant bitter and/or metallic taste that appear 1–2 days after ingestion and resolve within 5–14 days [1]. The cause of PNS is ingestion of pine nuts from the species of *Pinus armandii*. Pine nuts from the species of *P. armandii* are used industrially for non-food purposes and have entered the food chain because of mislabeling. Commercial edible nuts include the species of *P. cembra*, *P. koraiensis*, *P. sibirica*, *P. gerardiana*, *P. pinea*, *P. pumila*, *P. tabuliformis*, *P. wallichiana* (*griffithii*), and *P. yunnanensis*. The problems with *P. armandii* and PNS have showcased the need for authentication of pine nut species. In addition, mislabeling or fraud with other pine species might also occur as nuts from different pine species are differently prized.
Methods. Designed PCR primers target conserved DNA sequences that span an area of variation between *P. armandii* and the other included species. The variation included differences in single base pairs and in the length of amplicons. The high-resolution (hr) melting curve profiles were obtained from 4 unknown samples and 13 reference materials from 9 different species. Principal component analysis (PCA) of the hr-melting curve profiles was used to cluster pine species from the reference material, and determines the species from the unknown samples. A comparative study of the unknown samples investigated the validity of the method [2].
Results and discussion. The PCA successfully discriminated 2 subspecies of P. armandii, *P. gerardiana, P. pinea, P. tabuliformis, P. wallichiana (griffithii),* and *P. yunnanensis. Pinus koraiensis/ P. pumila and P. sibirica/ P. cembra* had identical DNA amplicons, respectively, and were discriminated in 2 distinct clusters. The unknown samples clustered together with P. armandii and P. koraiensis/ P. pumila, which was in agreement with results from the comparative species determination study. This demonstrated the applicability and validity of the developed method.
Perspectives. The presented strategy can discriminate more species from each other than traditional multiplex PCR. Furthermore, in contrast to DNA sequencing methods, our strategy is independent of DNA sequence libraries; important in this and other cases where DNA sequence data are scarce.

[1] Ballin, N. Z. (2012). A trial investigating the symptoms related to pine nut syndrome. Journal of Medical Toxicology, 8, 278-280.
[2] Aase Æ. Mikkelsen, Flemming Jessen, & Nicolai Z. Ballin. (2014). Species determination of pine nuts in commercial samples causing pine nut syndrome. Food Control, 40, 19-25.

Keywords: *authentication, pine nut syndrome, pinus armandii, principal component analysis (PCA), species determination*

B2
CHARACTERIZATION OF SOME OLIVE OIL QUALITY ASPECTS BY NIRS ANALYSIS OF ITS FATTY ACIDS AND TRIGLYCERIDES

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The development of new rapid techniques to characterize aspects of the quality of olive oils is of great interest, especially when they do not depend on the use of solvents and reagents. Two main areas of application of these techniques are 1) determining the varietal origin and 2) olive oil authentication against fraudulent mixtures of plant oils. In this work we have developed predictive models based on spectroscopy Vis/NIR that allow analysis of the composition of fatty acids in olive oil and accurately estimating their triglyceride composition. Strategies for developing fraud detection techniques on olive oils, based on the Vis/NIR analysis of the 'Equivalent Number of Carbon' (ECN), are also studied.

Keywords: *olive oil, fatty acids composition, triglycerides, varietal origin, fraud*

Acknowledgement: *To de project 'New strategies for olive oil characterization and detection of frauds', of the Plan Estatal de Investigación Científica y Técnica y de Innovación 2013-2016*

B3
AUTHENTICATION OF MILK SPECIES IN DAIRY PRODUCTS AT PROTEIN AND DNA LEVEL

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Dairy products made from sheep's and goat's milk are of considerable economic importance. The seasonal changes and the much lower milk yield of ewes and goats, together with the much lower price of bovine milk are the main reasons for this adulteration. However, the substitution of these milks for cheaper cow's milk is a fraudulent practice in dairy industry. As a consequence, an adequate methodology is required to control authenticity of dairy products. Moreover, dairy-like soy products, which are an alternative for people suffering from an allergy against milk proteins, have to be checked to prevent potential adulterations resulting from the addition of casein and/or whey proteins to these products and their adverse effects on allergic people. The objectives of this study were the qualitative detection and the quantitative determination of cow's milk percentage in dairy products. Standard mixtures of milk from different species as well as model cheeses of different ages were used as references. Species identification was performed using different electrophoretic methods, and by conventional PCR and quantitative real-time PCR using species-specific primers. Applied methods were evaluated regarding their applicability for the detection and quantification of cow's milk in mixed-milk cheeses and in soy products. Urea-PAGE of caseins was restricted to the adulteration control of milk and dairy products without any proteolytic changes, only. The official EU reference method, which is based on the IEF of gamma-casein fractions, was a reliable tool for the detection of cow's milk even in matured cheeses made from milk of other species. Moreover, after densitometric evaluation, a quantitative estimation of cow's milk percentage was obtained in mixed-milk cheeses. Urea–PAGE proved the method of choice to detect cow's milk in soy milk products, whereas IEF and SDS–PAGE of proteins were not applicable due to false-positive results. However, SDS–PAGE was the appropriate technique for the detection and semi-quantitative estimation of whey cheeses (e.g., Ricotta) that had been fraudulently added to other cheese varieties. PCR analysis was used to confirm the results of protein-based electrophoretic methods. Problems inherent in quantitative analysis of cow's milk percentage using protein-based techniques and even more using DNA-based methods were emphasized. Applicability of qPCR for the determination of cow's milk percentage in mixed-milk cheese was shown to be hampered by several factors (e.g., lack of information on the somatic cell count of milk; technological parameters influencing the final DNA concentration in ripened cheese samples). Thus, analytical procedures used were appropriate for the qualitative detection of cow's milk in dairy products. However, quantitative results in adulteration control have to be understood as approximate values, and authentication of mixed-milk cheeses still remains a challenge for food analysts.

Keywords: *dairy products, adulteration, milk species, electrophoresis, PCR*

B4
EXTRA VIRGIN OLIVE OIL: CARACTERIZATION AND CLASSIFICATION OF ITALIAN, EUROPEAN, TURKISH AND AMERICAN OILS ACCORDING TO GEOGRAPHICAL ORIGIN BASED ON UNTARGET APPROACH OF VOLATILE COMPOUNDS

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As defined by the International Olive Council, virgin olive oils are the oils obtained from the fruit of the olive tree (*Olea europaea L.*) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration. In particular, extra virgin olive oil (EVOO) is virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.8 grams per 100 grams, and the other characteristics of which correspond to those fixed for this category in the IOC standard. In terms of oil regulations, up to now, the EU has specified more than 100 protected denomination of origin (PDO) regions for olive oil and, in terms of oil industrial production and market (olive oil is a major constituent of Mediterranean diet), need to consider that one of the factors important for the consumers' choice is the geographic area of olive oil production. Using two independent systems (very simple and cheap in terms of sample preparation) was used to characterize and classify samples of olive oils (N=86; year of oil production: 2014) according to geographical origin based on analysis (untargeted approach) of volatiles compounds (N=63) with HS–GC/FID and SPME–GC/MS. Using chemometrics, raw, untargeted results were processed with multivariate analysis to generate a significant and reliable prediction model. After proper outliers removal (PCA for classes), a very high level of significance and reliability was reached with the PLS–DA model with R^2=0.80 and Q^2=0.74 and 100% of recognition ability.

Keywords: *extra vingin olive oils, geographic origin, mass spectrometry, untarget, chemometrics*

B5
CONSTRUCTION OF A LC-QTOF-MS LIBRARY FOR SCREENING OF 46 DRUGS IN DIETARY SUPPLEMENTS

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The sales cases of foods containing unauthorized substances have been increased worldwide, its safety management problems have been issued. It is needed to develop and utilize a MS library to analyze drugs in dietary supplements rapidly and accurately for the safety management. Therefore, identifying 46 drugs in dietary supplements by LC–QTOF–MS library searching are the purpose of this study. A liquid chromatography time of flight mass spectrometry (LC–QTOF–MS) is presented for the qualitative screening for 46 drugs in dietary supplements, which is considerably more than in previous methods. The Waters ACQUITY UPLC BEH C18 column (100 × 2.1 mm I.D., 1.7 μm) kept at 35°C in an oven was used. The mobile phases consisted of distilled water containing 0.1% formic acid (v/v, A) and acetonitrile containing 0.1% formic acid (v/v, B). These compounds were introduced into waters Synapt instrument equipped with electro-spray ionization (ESI) spray ion source operating in the positive or negative ionization mode. Identification was based on the compound's absolute retention time, protonated or deprotonated molecular ion, and fragment ion at an individually selected collision energy (CE). Based on retention time, the screening library was constructed including all 46 drugs. As a result, the advanced analytical method for 46 drugs in dietary supplements was developed and it contributes to establish a rapid and accurate library for searching unauthorized substances.

Keywords: *dietary supplements, drugs, LC–QTOF–MS, library, screening*

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B6
DETECTION OF FOOD ADULTERATION BY SYNCHRONOUS FLURESCENCE SPECTROSCOPY COMB INED WITH MULTIVARIATE DATA ANALYSIS

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Problem of food adulteration is dating back thousands of years. Methods of food adulteration detection have evolved over past few decades from the very simple to the very sophisticated and advanced analytical techniques. The aim of this study was to investigate the potential of synchronous fluorescence spectra measurements for the detection of adulteration of extra virgin olive oils, butters, rennet cheeses and chocolates. Experimental material consisted of samples of extra virgin olive oils, olive oils of lower quality, butters and plant oils, rennet cheeses, cheese-like products, chocolate and chocolate-like products. Fat extraction from cheese and cheese-like, chocolate and chocolate-like products was performed according to the Folch method. The model adulterant mixtures were constructed by spiking genuine fat with fraudulent fat at levels ranging from 0 to 100%, at 10% intervals (*w/w*). The synchronous fluorescence spectra of the samples and experimental mixtures diluted in n-hexane (1% *v/v*) were acquired within the excitation wavelength range of 240 to 700 nm for wavelength intervals of 10, 30, 60 and 80 nm. Successive projections algorithm (SPA) was applied to retain the most informative wavelengths from the spectra for further chemometric analysis. A multiple linear regression analysis (MLR) was applied to the previously selected fluorescence intensities (by SPA) of the experimental samples and their mixtures. MLR models were built separately for the data acquired at each wavelength interval (Δλ=10, 30, 60 and 80 nm). The root mean square errors of calibration (RMSEC) and the root mean square errors of cross validation (RMSECV) calculated by means of the leave-one-out method made it possible to asses and confirm the prediction ability of the models. Moreover limits of detection (LOD) of adulterants were calculated for the data obtained at selected wavelengths. The lowest RMSEC and RMSECV values were obtained at the wavelength interval of 60 or 80 nm and did not exceed 3 and 5%, respectively. The lowest detection limits (LOD) of adulteration for most products did not exceed 5% and were calculated for the measurements acquired at higher Δλ equaled 60 or 80 nm. The calculated LOD values and RMSEC/RMSECV for MLR models indicate the advantage of the higher wavelength intervals over the lower ones for adulteration detection.

Keywords: *food adulteration, fluorescence spectroscopy, chemometrics, limit of detection, multiple linear regression analysis*

B7
RAPID TARGET AND UNTARGET ANALYTICAL METHOD FOR ALKALOIDS ANALYSIS IN HERBAL EXTRACTS

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Alkaloids are a group of nitrogenous basic compounds commonly found in certain families of plants. Over ten thousand alkaloids have been isolated from nature resource [1]. Some of them are responsible for the beneficial effects of traditional medicines, but some may instead have harmful effects of poisons [2]. There is no uniform classification of alkaloids and, depending on their chemical structure, they can be classified into the following main groups: pyrrolidine, tropane, pyrrolizidine, piperidine, quinolizidine, indolizidine, pyridine, and isoquinoline structures. In particular pyrrolizidine alkaloids (PAs) have hepatotoxic, mutagenic, and cancerogenic effects [3] and in accordingly with the German Federal Institute for Risk Assessment (BfR) was identified for 1,2-unsaturated PAs a daily intake of 0.007 µg/kg body weight (0.42 µg/60 kg adult). Different studies revealed clearly that preparation of herbal infusions products may partly contain high amounts of PAs exceeding current recommendations [3,4]. A rapid screening untarget and target analytical method for alkaloids analyses using an UHPLC coupled with a Quadrupole/High-Resolution Mass Spectrometry (Orbitrap) was developed. The mass spectrometer operated in positive ion mode using the following parameters: sheath gas flow rate set at 30 arbitrary units; aux gas flow rate at 10 arbitrary units; spray voltage at 3.5 kV; capillary temperature at 330°C; aux gas heater temperature at 300°C; Mass spectra were acquired in full MS-data dependent MS/MS analysis (full MS–dd MS/MS) at mass resolving power of 140.000 and compared with a database of about 300 alkaloid molecules built from literature. A selection of commercial infusion extracts (mint, peppermint, fennel, aloe vera, chamomile, basil, almonds, lemon, passion fruit, black tea, white tea, green tea etc.) were studied in order to check the possible presence of a large number of alkaloids.

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Keywords: *alkaloids, infusion extracts, Orbitrap, mass spectrometry*

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B8
AUTHENTICATION OF FISH PRODUCTS BY MICROIMAGE TECHNOLOGY

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The production of seafood is approaching the all-time high of 160 million tonnes per year. Seafood remains a fundamental component of the human diet; for 3 billion people, it provides more than 20% of animal proteins consumed, it also remains an energetically favourable way to produce proteins, compared to farming terrestrial livestock. Yet, wild fisheries remain grossly mismanaged in many parts of the world and the seafood supply chain becomes increasingly difficult to regulate, as a result of the globalisation of traded commodities. Such a scenario is vulnerable to fraudulent operations, such as the deliberate mislabelling of seafood products. Seafood mislabelling opens the opportunity to fraud for economic gain but the economic gain can not exclude sanitary frauds. In this context a common fraudulent economic practice is selling valuable fish products as fresh when they have actually been frozen and thawed. The same mislabeling fraud could otherwise have an impact on human health, when fish intended for raw consumption is administered fresh instead of frozen, as expected from Regulation EC 853/2004 and Regulation EU 1276/2011, to reclaim parasites and to prevent human infestation. This presentation is focused on perspectives of the application of a new rapid, cost-effective and high throughput method that allows to discriminate between fresh and frozen-thawed fish in the frame of monitoring plans for fish authentication. Automation development as an official routine screening tool fulfilling the EU requirements stated in the EC Regulation No. 882/2004 will be discussed.

Keywords: *fish, fraud, fresh, thawed*

B9
CLASSIFICATION OF EXTRA VIRGIN OLIVE OILS OF THE TWO MAJOR CYPRIOT CULTIVARS BASED ON THEIR FATTY ACID AND VOLATILES COMPOSITION

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There is no data concerning the fatty acid composition of Cypriot extra virgin olive oil. Thus, seventy-four authentic samples of Cypriot extra virgin olive oils were collected from various locations over two years harvest. The two dominant Cypriot olive cultivars, Cypriot (ladoelia), and Koroneiki (lianolia) were analysed for fatty acid composition by GC–MS. Fatty Acid Methyl Esters (FAME) were prepared from olive oil, using a cold saponification method. Volatiles composition was measured by GC–MS using the SPME method. The data obtained were analysed statistically using Principal Component Analysis aiming for classification of oils, and one-way analysis of variance, for comparing the mean between varieties or locations. Statistical analysis showed that the olive oils could be separated into distinct groups, using the FAME data bank obtained. It was concluded that the method showed considerable potential for the classification of olive oils, according to both the botanical and the location of origin.

Keywords: *EVOO, FAME, GC–MS, SPME, volatiles*

B10
DO WE KNOW WHAT'S REALLY IN OUR FOOD? FOOD FRAUD AND ADULTERATION, HOW CAN WE DETECT IT

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Detection tools are vital to control and deter food authenticity issues. With a need to test more samples and the challenge of ever more insightful fraudsters that look to outwit existing testing techniques, rapid tests that screen for a broad range of fraudulent activities are becoming more widely adopted. This presentation will focus on new technologies that enable samples to be tested in less than a minute. Several examples will be included in high risk ingredients such as dairy, spices and olive oils.

Keywords: *TOF MS, food fraud, authenticity, FTIR*

B11
VALIDATION AND REPORTING STANDARDS OF NON-TARGETED FOOD FINGERPRINTING APPROACHES

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Food fingerprinting approaches are typically based on a high throughput screening of samples with the purpose of their comprehensive characterisation as well as differentiation/classification. After determination and mapping of the patterns of individual food or feed matrices, the aim is to differentiate various analytical fingerprints/samples in terms of e.g. their botanical or geographical origin on the one hand or with respect to possible forbidden additions on the other hand. These approaches have been demonstrated to be successful in many research studies investigating the authenticity of various food and feed matrices, but their application in routine analysis or implementation in regulatory frameworks is still in its infants. Whereas for classical targeted analytical procedures used for routine analysis and regulatory purposes usually validation and protocols do exist, for non-targeted approaches, typically based on spectroscopic or spectrometric measurements and a subsequent multivariate statistical data evaluation, such strategies have not yet been developed. A review of literature with regard to validation strategies, for food authentication using metabolic fingerprinting techniques, revealed two issues that may hinder a broader application of such metabolomics approaches: a lack of validation strategies for the metabolomics workflow and limited reporting on data quality [1]. In particular, model validation within the experiment was well addressed, but the accuracy of the results considering experiment-to-experiment, instrumental, operator or laboratory variation was hardly considered. Moreover, data processing was little transparent in many studies with poorly characterised metabolic fingerprints used for data evaluation. On this basis, recommendations and future work for validation as well as reporting of non-targeted food fingerprinting are derived to progress standardisation for a potential implementation of such approaches in routine analysis for food control purposes. These suggestions of “good practice” for validation and reporting might also be relevant for further applications within regulatory frameworks.

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Keywords: *non-targeted analysis, authentication, standardisation, chemometrics*

B12
Cancelled

B13
A COMPREHENSIVE STRATEGY TO DETECT THE FRAUDULENT ADULTERATION OF OREGANO

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Fraud in the global food supply chain is becoming increasingly common due to the huge profits associated with this type of criminal activity. Food commodities and ingredients that are expensive and/or are part of complex supply chains are particularly vulnerable. Herbs and spices fit these criteria perfectly and yet strategies to detect fraudulent adulteration are still far from robust. Oregano is a culinary herb most commonly associated with pizzas and other Mediterranean dishes. The main producers of oregano reside in the USA, Mexico, Greece and Turkey. The industry gold standard for testing for adulteration is a microscopy based method. The aim of this study was to develop and fully validate a two-tier approach utilising Fourier-Transform Infrared spectroscopy (FTIR) and Liquid Chromatography High Resolution Mass spectrometry (LC–HRMS) to screen for and confirm oregano adulteration with common adulterants e.g. olive leaves, myrtle leaves. When these two techniques are combined with multivariate data analysis software they have the ability to screen and process a large number of samples. The two tier testing strategy was applied to a 78 sample survey obtained from a variety of retail and on-line sources. There was 100% agreement between the two tests that over 24% of all samples tested had some form of adulterants present. The innovative strategy devised could be used as a basis for testing the global supply chains for fraud in many different forms of herbs.

Keywords: *oregano, fraud, FT-IR, QToF-MS, chemometrics*

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B14
SECONDARY PLANT SUBSTANCES FOR THE CHARACTERIZATION OF RASPBERRY HONEY

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Monofloral honeys are high-quality honeys and due to their characteristic flavor, quite popular among consumers. The monofloral honeys are named after the generic type of the plant. Melissopalynological and physicochemical properties are employed for the differentiation between these honeys. In addition, their phenolic and volatile compounds proved promising for differentiating between their botanical origins. In the present study, raspberry honeys (Rubus idaeus) were investigated. The characterization was carried out by analyzing the volatile compounds via gas chromatography (GC) after headspace solid-phase micro-extraction (HS–SPME). Furthermore, non-volatile secondary plant substances were determined by solid phase extraction combined with UHPLC–DAD–MS/MS (Oelschlägel et al. (2012), J. Agric. Food Chem., 60: 7229–7237). In the phenolic extracts of the raspberry honeys, large amounts of ellagic acid were identified. Therefore, ellagic acid is a suitable floral marker to distinguish raspberry honeys from 14 other European monofloral honeys, for example acacia, buckwheat, cornflower, lavender, and rosemary. Regarding the volatile profile of raspberry honey, the major compounds were nonanal, nonanol, and beta-damascenone. Furthermore, we analyzed the nectar of raspberry flowers in order to get additional information regarding the alteration of the compounds during the honey production.

Keywords: *honey, rubus idaeus, secondary plant substances, UHPLC–DAD–MS/MS, HS–SPME–GC–MS*

B15
NMR-PROFILING OF HONEY – THE NEW APPROACH IN HONEY AUTHENTICITY TESTING

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In recent years, adulteration of honey, for example by addition of syrup, has become an important issue. Classically, testing for adulteration mostly focuses on the detection of certain marker compounds. However, the absence of marker compounds does not necessarily mean that a particular honey is not adulterated. Moreover, production of syrups used for adulteration is changed gradually to prevent the formation of marker compounds that are typically targeted by conventional methods. Thus, conventional methods may get bypassed. The aim of this project was to develop a more comprehensive method to confirm the general authenticity of honey. This includes the botanical and geographical authenticity as well as the detection of adulteration, for example by addition of sugar. By combining targeted and non-targeted analyses the method may also detect new or so far unknown types of adulteration, which conventional analysis would not detect. Quality Services International GmbH (Bremen, Germany) is part of a consortium with Bruker BioSpin GmbH (Rheinstetten, Germany) and ALNuMed GmbH (Bayreuth, Germany) to develop honey analysis to a completely new performance standard. A database of authentic reference honeys is built, and multivariate statistics are used to check unknown samples for authenticity. Over 4,000 samples from more than 45 countries have been analyzed by QSI and ALNuMed GmbH and form part of the first product release. Not only polyfloral honeys were sampled, also honeydew and various monofloral honeys were collected to be included in the database. Authenticity of honey samples was supported by a range of conventional methods, including pollen analysis, analysis of 13C-ratios, detection of foreign enzymes, and others. Moreover, a variety of syrup samples was collected as well, and adulteration experiments with different syrups were conducted. The FoodScreener TM developed by Bruker BioSpin GmbH for food analysis is a standardized platform for 400 MHz Nuclear Magnetic Resonance (NMR) spectroscopy for the analysis of various food matrices. The principle is based on the acquisition of the spectroscopic fingerprint specific for each individual sample. For juice and wine it is already a well-proven concept, which is now being established as a screening method to confirm honey authenticity and perform quality control. Combining targeted (quantification) and non-targeted (statistics for classification and verification) approaches, it becomes possible to address previously unanswered questions and allow simultaneous identification and absolute quantification of a multitude of relevant parameters (sugars, acids, amino acids, …) and the development of statistical models for the analysis of authenticity. This approach allows the detection of adulteration, for example by addition of sugar syrup, as well as verification of botanical or geographical origins.

Keywords: *honey, authenticity, adulteration, fraud, NMR*

Acknowledgement: *Many thanks for their valuable contributions and discussions to the colleagues from ALNuMed GmbH (Stephan Schwarzinger and Paul Rösch) and Bruker Biospin GmbH (Fang Fang, Birk Schütz and Manfred Spraul).*

B16
AUTHENTICATION OF HARD CHEESE BASED ON NMR FATTY ACID PROFILING

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The cheese has high nutritional value, to some extent because of their fat composition [1]. The use of ^{1}H-NMR spectroscopy in authentication studies of various food products proved to be a valuable tool [2–5]. The aim of this work is to authenticate various hard cheeses using a quick and reliable method: NMR spectroscopy combined with chemometrical computation. In this study have been analysed fats from hard cheese. The selected hard cheese samples were produced in Romania; some traditional products (Rucar, Dalia and Penteleu) have been taken in consideration. The fats extracted from various sources are directly analysed by NMR spectroscopy. In order to obtain the fatty acid composition of those products, the spectral NMR data has been subjected to a set of computational equations established in this research. In terms of fatty acid composition few relevant classes have been identified such as: short chain and long chain saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. The fatty acids composition of samples was confirmed by GC–MS standardized method. The samples have been analysed as well by FT–IR spectroscopy, spectral information being used in authentication studies. The samples were discriminated by several criteria, such as type of product, area of origin and type of feedstock. This is very important in the context of the new European Union's common market with a wide range of products and a high demand for qualitative products, with great importance especially for traditional products with geographical protected origin.

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Keywords: *authentication, hard-cheese, milk based products, NMR spectroscopy*

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B17
DETERMINATION OF UNAUTHORIZED SUDAN RED AZO-DYES IN TOMATO SAUCE, CHILLI POWDER, AND PALM OIL BY DIRECT SAMPLE ANALYSIS IN HIGH RESOLUTION MASS SPECTROMETRY

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Sudan Red azo-dyes are normally used to colourize waxes, oils, petrol, solvents and polishes. They are categorized as class 3 carcinogens by the International Agency for Research on Cancer and therefore are illegal as food additives according to both FDA and the EU. Since 2003, European nations have required random product testing and testing of suspected adulterated products, with a detection limit (LOD) of 0.5–1 mg/Kg. Due to the continue illicit use of Sudan Red dyes as food colorants, their determination in different food matrices, especially in chilli powder, tomato sauces and palm oil, has received during the recent years increasing attention all over the world. Thus it is of particular interest the availability of analytical methods that are sensitive and fast both to protect consumers from frauds and to not excessively interfere with the distribution chain needs. In the present study we developed an easy and fast method to extract and detect Sudan Red dyes in different matrices, by Direct Sampling Analysis (DSA) coupled with a High Resolution Mass Spectrometer (AxION2 TOF - Perkin Elmer). Stock solutions (1 mg/ml) of Sudan I, II, III and IV (Sigma Aldrich) were prepared in ethyl-acetate. A tomato sauce, a chilli powder and a palm oil in which Sudan dyes were demonstrated to be absent were considered as the blanks and used to prepare samples singly spiked with the four Sudan dyes from 0.1 to 10 mg/Kg. Furthermore 40 samples of various matrices, coming from local market and five samples previously examined by LC–MS by another laboratory and founded positive for at least one Sudan dye, were analysed. 10ul of each extracted sample were pipetted directly onto the stainless mesh of the AxION DSA for ionisation and analysis. Measurement was run in positive ionization mode with flight voltage of -10,000V. Mass spectra were acquired in a range of m/z 100–2,000 at an acquisition rate of 1 spectra/s. To obtain higher mass accuracy, calibration solution was infused into the DSA source at 10 µl/min. The whole analysis of one sample took two minutes. Mass spectra of the four dyes with or without matrix at the examined concentrations were all identified. The detection limit (LOD), evaluated as the minimum concentration of analyte that provides a spectrum in which an exact mass measurement is feasible, was 0.05 mg/Kg for each Sudan dye solution. The error in mass identification was lower than 5 ppm for each concentration of Sudan dye solution. Recovery obtained for each dye, calculated on spiked tomato sauce, ranged from 90% to 105% at concentration of 1 mg/Kg and 0.5 mg/Kg. None of the commercial samples examined was found positive for Sudan dyes while we were able to confirm the contamination of the five positive samples. In conclusion, the applied method was able to reveal the presence of Sudan Red dyes in real and in spiked samples at concentrations that are consistent with the level indicated by the EU.

Keywords: *high resolution mass spectrometry, Sudan red dyes, food fraud*

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B18
BASMATI OR NOT BASMATI? THAT IS THE QUESTION

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There is increased concern on the authenticity of basmati rice sold throughout the world. There is reason to believe that economic adulteration, of the commodity being imported from India, is occurring. According to many reports, basmati rice is being adulterated with the cheaper, easier to grow and less aromatic rice More and more sophisticated techniques are required to detect food fraud across a range of commodities that demand a higher price than their cheaper alternatives. A proof of principle method has been established to assess the authenticity of basmati rice using 'off the shelf' supermarket samples with the latest advancements in high resolution GC–MS hardware and informatics. Data independent, information rich, HDMSE acquisitions enable accurate mass precursor and product ion information to be captured in a single injection. The combination of a GC separation, a HDMSE acquisition and informatics able to process and review four dimensional data resulted in a unprecedented level of specificity for all compounds detected in the chromatographic run. This is demonstrated when displaying precursor and product ion spectra that have been drift time and time aligned. Alignment algorithms within the statistical analysis package enabled features across injections to be properly aligned prior to peak picking which improved the ability to track ions of interest across all injections. Models within the software such as PCA, OPLS–DA and Correlation Analysis allowed ions of interest in complex matrices to be isolated efficiently and accurately. The integration of several publically available databases into the processing method made preliminary identification of significant ions identified by the statistical analysis facile and automated Automatic calculation of collision cross section (CCS) values for all detected compounds was performed using the drift times obtained from the ion mobility separation. These CCS values can be added to database entries for use as an additional level of selectivity in future experiments. A proof-of-principle method for the investigation of basmati rice authenticity and potential food fraud was devised and will be discussed.

Keywords: *rice authenticity, APGC, ion mobility*

B19
RAPID SCREENING FOR FOOD ADULTERATION USING MID- AND NEAR-INFRARED SPECTROSCOPY

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Economically motivated adulteration (EMA) of food has become increasingly prevalent in recent years as a result of manufacturers desiring increased profit margins. However, EMA can negatively impact consumer confidence, and more importantly some adulteration can be detrimental to health. Compared to the often trace levels involved in accidental contamination, adulterants are usually added in substantial quantities in order to ensure greatest profit gain. Near-infrared (NIR) and Mid-infrared (MIR) spectroscopy are valuable techniques for the rapid screening of food products in order to confirm the quality of the product and any potential adulteration at relevant concentrations. These techniques require little or no sample preparation at all. Existing targeted approaches for adulterant screening require a quantitative calibration to be developed for each potential adulterant. Non-targeted screening approaches can determine when there is a potential adulteration problem but can neither identify nor quantify the adulterant. The powerful Adulterant Screen™ combines the advantages of both targeted and non-targeted approaches, allowing easy detection and quantitative estimation of adulteration at relevant levels. A range of examples of food adulteration studies using MIR and NIR spectroscopy demonstrates the use of Adulterant Screen as a rapid screening method for suspect materials.

Keywords: *infrared, spectroscopy, adulteration, food safety, screening,*

B20
WORKFLOW DEVELOPMENT FOR THE NEAR-INFRARED PREDICTION OF FOOD COMPOSITION AND DETECTION OF ADULTERATION

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Increasing legislation and consumer concerns regarding food safety has led to a significant increase in the amount of testing performed. Near-infrared (NIR) spectroscopy is an analytical technique that is used extensively throughout the food industry performing raw materials testing all the way through to the testing of final products. NIR spectra contain a wealth of information and can provide both qualitative material identification and quantitative compositional analysis including protein, fat and moisture contents with a single rapid measurement. A food testing regime requires a multi-step process or workflow. Firstly, it is necessary to check that the material is what it is claimed to be and is of the correct quality. This can be achieved using a non-targeted approach, such as SIMCA, to simply provide that confirmation. After confirming that it is the correct material and quality it is necessary to confirm that the material is not contaminated or adulterated. This is achieved using the powerful Adulterant Screen™ algorithm, a semi-targeted chemometric method, allowing detection and quantitative prediction of potential adulterants within a material. Finally, quantitative analysis of food composition can be achieved using Spectrum® Quant by building a library of standards of known compositional values and generating a Partial Least Squares (PLS) calibration. All of these steps can be simply combined in a powerful workflow for complete testing of the food product. The workflow is implemented in a stepwise click (or touchscreen) interface perfect for routine QC analysts. The software reports PASS/FAIL results confirming the material is the correct material, whether the material is adulterated and if the components are within the concentration limits expected. An example of the NIR analysis of a powdered food product is demonstrated.

Keywords: *near-infrared, spectroscopy, adulteration, authenticity, screening*

B21
RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (REIMS) FOR FOOD AUTHENTICITY TESTING

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The quality, safety and authenticity of food are of principle interest for society and are regulated by legislation. Food fraud is used to encompass deliberate and intentional substitution, addition, tampering, or misrepresentation of food, ingredients, packaging or false statements for economic gain. Due to their high market value, meat and fish products are often targets for species substitution, adulteration, mislabelling and questions raised about geographic origin or means of production. Absence of a declared species or presence of an undeclared species would raise doubts about the claimed provenance of the product and some cuts of meat are more valuable than others. Testing food is one of the key ways of checking whether food businesses are complying with food law. Current methods used for determination of species and adulteration (e.g. ELISA, genomics, chromatography, spectroscopy or mass spectrometry) are time consuming, can be costly and typically located in a laboratory some distance from the producer and retailer. Rapid Evaporative Ionisation Mass Spectrometry (REIMS) combined with multivariate statistical analysis (Principal Component Analysis and Linear Discriminate Analysis) is an emerging technique for near real time characterization of tissues with no requirement for sample preparation [1,2]. Samples are analysed by direct cutting of the surface of the sample using hand-held sampling devices powered by an electrosurgical RF-generator; a monopolar cutting electrode (the iKnife) or bipolar forceps. The resulting “smoke” or aerosol generated is transferred to the mass spectrometer via a Venturi air jet pump-based ion transfer apparatus mounted in the orthogonal position relative to the atmospheric interface of a quadrupole time of flight mass spectrometer. Although mass spectra acquired from food samples, including a range of different fish species or from different cuts of meat, look similar, the profile of the lipid components has been shown to be useful for classification purp oses using multivariate statistical methods. Using these spectra, training samples were used to classify the reference groups to build PCA/LDA models. The models were verified with cross-validation and independent test sets. We present preliminary data that demonstrates the potential capability of REIMS technique to accurately discriminate samples from different species, for distinguishing cuts of meat and for the analysis of other food types (e.g. honey and coffee).

[1] J. Balog et al., Analytical Chemistry, 82, 7343-50 (2010)
[2] J. Balog et al., Science Translational Medicine, 5, 194ra93 (2013)

Keywords: *food authenticity, speciation, adulteration, REIMS, i-Knife*

B22
FT-IR FINGERPRINTING TO IDENTIFY MINERAL OIL ADULTERATION IN SUNFLOWER OIL

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Authentication of edible oils, focusing e.g. on the verification of the respective geographical/ botanical origin, production processes and/or the determination of forbidden additions, is a relevant issue in industry and official food control. Adulteration of olive oil for example reveals at one of the first places of commodities in actual compilations of incidents related to food fraud [1]. Blending of olive oil with seed oils or different qualities is one common problem for example, which affects the quality of the product and often results in a substantial economic damage. However, also other adulterations of edible oils have been discovered and reported. An example is the notification of the Rapid Alert System for Food and Feed (RASFF) in 2008, concerning unrefined sunflower oil originating from Ukraine, which was found to be contaminated with high levels of mineral oil. The origin of the contamination was never finally clarified but it has been speculated that intentional adulteration was the reason for these high levels of mineral oils. Aim of the presented study was to investigate the applicability of Fourier Transform Infrared (FT–IR) spectroscopy in context of the non-targeted detection of mineral oil adulteration in sunflower oils. For this, a set of commercially obtained sunflower oil samples of different brands as well as experimental mixtures of sunflower oils and additions of several types of mineral oils (in eleven concentration levels) were analyzed using Attenuated Total Reflection FT–IR spectroscopy. The acquired data set was used in a first step to identify a suitable data pre-processing. Further Partial Least Squares (PLS) –Regression models were generated and validated by Monte Carlo cross-validation and independent test sets in order to assess the limit of detection of mineral oil in sunflower oil using FT–IR spectroscopy.

[1] Moore et al. Journal of Food Science 2012, 77, R118-R126.

Keywords: *non-targeted analysis, edible seed oil, authenticity, FT–IR, adulteration*

B23
CHEMICAL PROFILING OF WHISKIES USING ORBITRAP GC–MS

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Whisky is a premium distilled spirit beverage that is produced using long established methods that involve a complex aging process. These processes result in a final product that has unique characteristics, has high commercial value, and can be economically important in the regions of the world where it is produced and consumed. As such, it is essential that whisky producers are able to obtain an accurate and comprehensive chemical profile which is characteristic to their individual product. This information can then be used to identify adulterated or counterfeit products and enable action to be taken, with confidence, to protect the product and brand. Gas chromatography coupled to mass spectrometry represents one of a number of different analytical approaches that can be applied to meet this objective. It is important to consider both the sample preparation protocol employed and the parameters used in the instrumental analysis. In this study, we used GC–Orb itrap mass spectrometry for profiling components contained in ethyl acetate extracts prepared from several whiskies differing in origin and age. The key objective of this 'proof of concept' study was to analyse samples from different geographical regions and ages and to demonstrate the potential of the Thermo Scientific Q Exactive GC system to provide comprehensive information on the occurrence of both low and high intensity components, which is needed for sample classification. Non-target full range high resolution mass data was acquired and then evaluated using accurate mass deconvolution and spectral matching along with sophisticated statistical tools to detect any chemical differences between the samples. The measurements were performed in EI full scan (*m/z* 50–600) at a mass resolving power of 60,000 (FWHM, *m/z* 200). In addition to the experiments above, each sample was analysed singly using positive chemical ionisation to obtain molecular ion information to further support the proposed identity of unknown component peaks. The data was deconvoluted to extract components present at low and high concentrations and to provide clean spectra. The cleaned spectra were then searched against nominal mass spectral libraries and further filtered based on accurate mass matching of the proposed hits. The sub 1 ppm mass accuracy, across peaks of different intensity, enabled proposed compound identities to be quickly confirmed or eliminated. The combination of EI and PCI spectra provided information for the identification of unknown peaks in the chromatogram. The complete chemical characterisation of both low and high intensity components provided a comprehensive profile of the whisky samples. Further details will be presented in the poster.

Keywords: *chemical profiling, orbitrap mass spectrometry, accurate mass, whisky*

B24
DART APPLICATION FOR ANALYSIS OF CIGARETTE PACKAGES

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The Direct Analysis in Real Time (DART®) ion source is a relatively new mass spectrometry technique that is seeing widespread use in chemical analyses world-wide. DART is an ambient ionization technique which allows the analysis of complex solid samples with no or minimal sample pre-treatment by simply placing them between a DART ion source and a mass spectrometer. A method for rapid analysis of cigarette packaging without sample processing was explored by fingerprint identification of ink and lacquer constituents using a quadrupole TOF mass spectrometry (Q-TOF–MS) soft DART ionisation technique. In the present study a comparison of genuine and counterfeit samples of three cigarette brands (Red Point, B&H Gold, Viceroy Red) was made. This experiment was conducted using DART® SVP coupled to a Bruker Impact HD™ Q-TOF HRTOFMS instrument. The method used $[M + H]^+$ ions in positive mode in mass range of 50–900 *m/z*. Data obtained show the potential for an efficient high throughput strategy for authentication of cigarette packages using a rapid screening technique that can readily differentiate between the authentic package and other counterfeit products. However, method sensitivity and quantitation require further study and improvement.

Keywords: *DART, direct analysis in real time, cigarette packaging*

B25
DEVELOPMENT OF METAGENOMIC METHODS FOR DETERMINATION OF ORIGIN OF OYSTER SAMPLES

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The contamination of beef products with horsemeat in 2012 brought food authenticity to the attention of consumers. This, together with the use of quality labels (e.g. Red Tractor scheme) and EU regulations (PDO and PGI designations) strengthen the need for methods that can verify geographical origin (GO) labelling claims. Despite this, there are few techniques for the verification of the GO of food commodities, with stable isotope analysis being the current gold standard. Microbial community profiling may be a good way to determine GO since all products carry their own microbial community which can be influenced by factors including location and processing techniques (Le Nguyen, 2008). DNA sequencing techniques, for example next generation sequencing (NGS), are now sufficiently advanced to be able to generate a non-targeted and complete picture of total microbial communities associated with a given product, the microbiome. Analysis of this microbiome may provide data for GO designation. The work presented here focused on the use of NGS on Illumina's MiSeq platform for the identification of the bacteria associated with oyster gills using both RNA and DNA based approaches. RNA and DNA extracts of oyster gills were prepared and analysed in parallel using methods which analyse the whole microbial community associated with a sample: metabarcoding studies the 16S gene of all bacteria in one sample whereas metatranscriptomics and metagenomics both study all the genetic material recovered directly from the sample. Their capability to produce a dataset from the total oyster microbiome was assessed. It was found that of the metabarcoding approaches, one fragment was too large for use on the MiSeq, which resulted in a dataset which was too small to be useful. The other metabarcoding approach gave a good level of detail and was found to give a larger dataset, with a higher number of taxa found in the DNA compared to the RNA based dataset respectively. The data from the metatranscriptomics and metagenomics approaches contained large proportions of eukaryotic DNA, originally derived from oyster and associated parasites. Consequently the size of the microbial dataset obtained was much smaller than using metabarcoding approaches. It was therefore concluded that the DNA metabarcoding technique was the most cost effective and informative microbiome profiling technique of those tested and further studies are ongoing to determine the efficacy of this approach for the discrimination of GO for oysters.

[1] Le Nguyen, D., et al., (2008). Food Control 19:454–460

Keywords: *authenticity, origin, sequencing, metagenomics, metabarcoding*

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B26
INTEGRATING MICROSATELLITE (SSR) AND SINGLE NUCLEOTIDE POLYMORPHISM (SNP) DNA MARKER DATA TO ASSES TRACEABILITY IN CHILEAN BLUE MUSSEL (MYTILUS CHILENSIS)

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Marine mussels of the Mytilus genus are one of the most cultivated and marketed bivalves appreciated as a nutritious and tasty food source, and widely used in prepared food products in many countries. The international seafood trade has adopted standards and regulations intended to ensure food quality, safety and authenticity along the food chain -"from farm to fork"-, these include requirements related to follow the movement of material through all stages of production, processing and distribution, increasing the need for reliable traceability systems. The current food labeling system is not failure free and requires validation through analytical procedures. Therefore, DNA-based methods developed for species identification and population genetics, coupled with allocation algorithms can be used to verify administrative traceability systems. We evaluated the assignment power of panels including microsatellite (SSR), single nucleotide polymorphisms (SNP) a nd both types of DNA markers, to assign Mytilus individuals from southern Chile to their geographical origin: location and zone. We genotyped 190 individuals collected from six locations in Southern Chile, grouped in three different zones (1-Reloncaví, 2-Chiloé and 3-Magallanes), with different datasets: i) 14 SSR, ii) 1280 SNP–Total, iii) 981 SNP–Neutral, iv) 58 SNP–Outlier, v) 34 SNP–Outlier and vi) 24 SNP–Outlier, and combinations of i) with each of the SNP datasets (ii to vi), testing a total of 11 marker panels. For comparison of the assignment power of the different datasets, re-allocation tests were performed with the leave-one-out procedure in GeneClass2 considering three scenarios: 1) All the six locations from the three zones, 2) Five locations from 1-Reloncaví and 2-Chiloé zones and 3) Four locations from 1-Reloncaví zone. Datasets that include SSR and SNP markers re-allocate individuals to zone equal or better, than SSR or SNP marker panels separately. At a finer-scale level in the re-assignment of individuals to location, combined datasets always performed better than independent SSR or SNP marker panels. The Highest performance re-allocating individuals to zone was obtained with the 1240 SNP–Total and the 58 SNP–Outliers panels alone, and combined with the 14 SSR dataset, but in re-assignment of individuals to location, the 14 SSR – 58 SNP-Outliers and the 14 SSR – 24 SNP-Outliers panels performed best. On the base of these results we recommend the use of the panel with 14 SSR – 58 SNP-Outliers to perform traceability in southern Chile.

Keywords: *traceability, mytilus, microsatellite, SNP, assignment*

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B27
CORNFLOWER HONEY – A CASE FOR FLUORESCENCE

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Monofloral honeys are known for their color, consistency, and especially for their typical smell and aroma. Therefore, they clearly achieve higher prices on the market. This also includes the monofloral cornflower honey, which is used for wound healing due to its high content of hydrogen peroxide along with an antibacterial activity [1]. To classify and differentiate the cornflower honeys from other monofloral honeys, a UHPLC–PDA–MS/MS method was developed which analyzes and quantifies the cornflower marker lumichrome next to phenols and flavonoids [1,2]. However, the method requires a high equipment expenditure. For this, a simple TLC method was developed and validated using the blue fluorescent properties of lumichrome. Samples wrongly declared as cornflower honeys can be indicated and sorted out by comparison of the fluorescence intensity to a defined lumichrome standard. The screening method is able to analyze ten honey samples within two hours. Therefore, a honey solution (25%) is used directly without cleanup for TLC. All of the 13 cornflower honeys were in accordance with their declaration. The method is suitable for the specific screening of cornflower honeys with simple and easy application.

[1] S. Oelschlaegel et al., J. Agric. Food Chem. 2012, 60, 11811–11820.
[2] N. Beitlich et al., Lebensmittelchemie. 2014, 68, 110–111.

Keywords: *monofloral cornflower honey, lumichrome, fluorescence, TLC*

B28
SPECTROSCOPIC TECHNIQUES FOR DETECTING ADULTERATION OF BRITISH AND IRISH COLD PRESSED RAPESEED OILS

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The aim of this research is to construct a quick and accurate spectroscopic screening method which is able to detect when cold pressed rapeseed oil has been adulterated with either refined rapeseed oil or refined sunflower oil. Cold pressed rapeseed oil is marketed as a high quality product and consequently its retail value is at the top end of the edible oil market. There is no evidence that adulteration has occurred in the cold pressed rapeseed oil market. However this does not mean that the subject should not be investigated. High value edible oils are an easy target for adulteration as they can be mixed with cheap edible oils without greatly changing either the taste or the appearance of the original oil. To secure the burgeoning cold pressed rapeseed oil industry from any future threats it would be sensible to develop a screening technique capable of quickly and accurately detecting adulterants. The two types of spectroscopy used in this investigation w ere Raman Spectroscopy and Fourier Transformed-Infrared Spectroscopy (FT–IR). Comparing the two techniques gave an indication as to which would be more suitable for cold pressed rapeseed oil authentication. Two calibration sets were constructed, one contained refined sunflower (n=10), cold pressed rapeseed oil (n=14) and mixtures of the two (n=32). The other calibration contained refined rapeseed oil (n=10), cold pressed rapeseed oil (n=14) and mixtures of the two (n=32). These calibration sets had corresponding validation sets which were approximately a third smaller in size. The results show that the most successful technique for detecting refined rapeseed oil in cold pressed oil was Raman spectroscopy coupled with PLS–DA analysis. The most accurate technique for detecting sunflower oil in cold pressed rapeseed oil was FT–IR coupled with PLS–DA analysis.

Keywords: *cold pressed rapeseed oil, authentication, fraud, spectroscopy*

B29
ARE PORK RESIDUES PRESENT IN MY GUMMY BEARS? GELATIN SPECIATION BY LC–MS/MS

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Following the Food Standards Agency (FSA)'s announcement in January that horse and pig DNA had been identified in beef products sold by several supermarket chains, further testing across Europe and beyond has revealed widespread incidences of such food contamination. This intended adulteration for financial gain or careless false declaration of meat products is a severe problem for consumers who have ethical or religious concerns about the consumption of pork or horse, more specifically the Muslim or Jewish communities who represent about 23% of the worldwide population. As the tolerance level for porcine and equine content in foods is 0%, for religious reasons, the limit of detection (LOD) needs to be as low as possible and so the continued development of more sensitive methods is necessary. However, pork based products are not only used as the meat but can also be found in gelling agents in food (for example in candy, ice cream, and marshmallows) as well as in the cosmetic and pharmaceutical industry in the form of gelatin. Gelatin is made from collagen, a protein, which has been extracted from the skin, bones, and connective tissues of animals such as cows, chicken, pigs, and fish. Here we present the results from the initial development of an LC–MS/MS method utilizing AB SCIEX TripleTOF® 5600 and QTRAP® 4500 LC/MS/MS systems for the determination of the origin of gelatin used in food products and also pharmaceutical capsules.

Keywords: *meat speciation, authenticity, LC–MS/MS, pork, horse*

B30
PROVING THE AUTHENTICITY OF HONEY FOCUSED ON THE NATIVE AND FOREIGN ENZYMES

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Honey is an interesting and traditional food with unique nutritional and sensory properties. Recently in the Czech Republic there has been a lack of quality honey mainly due to adverse weather conditions and large mortality of honey bee colonies (fall 2014 / spring 2015, a 35% decrease of bee colonies). In the market adulteration of honeys is increasing, e.g. authentic honey mixed with sugar syrups or bee feeding by these syrups. In this study the methods generally used for the proof of enzymes present in honey were verified and critically evaluated. Diastase belongs to the most frequently analyzed enzymes for which minimum values are defined by legislation. Diastase activity is caused only by the presence of α-amylase (EC 3.2.1.1). Other amylases, i.e. β-amylase (EC 3.2.1.2) or γ-amylase (EC 3.2.1.3), are not found naturally in honey. Residual activities of β- or γ-amylase may originate from syrups used for bees feeding or these syrups are directly added into honey. α-amylase, known as an sign of honey freshness, is closely associated with a high content of 5-hydroxymethylfurfural (heat treatment indicator). However, cases of α-amylase addition into honey for a purpose of diastase activity increase are also known. Amylase activity was determined for a set of authentic and suspicious honeys during our study; also model samples prepared by the addition of enzymes (α-amylase, β-amylase, γ-amylase and baking mixture of amylases) with known activity into authentic honey were analyzed. The following methods were applied: Phadebas method, Shade method, the enzyme kit for the determination of β-amylase and the Shade method modified for analysis of thermostable α-amylase activity. The result showed that the evaluation of honey quality by traditional Shade and Phadebas methods is currently insufficient because the methods do not detect all forms of honey adulteration, i.e. presence of foreign amylases.

Keywords: *amylase, diastase, honey, authenticity, foreign enzymes*

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B31
AUTHENTICITY OF THE THYME HERB? THE POLYPHENOLS GIVE THE ANSWER

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Thyme is a widely used culinary herb. Its volatile compounds considerably influence its smell and taste. Consequently, they are an important criterion in the quality control of thyme. In 2013, a routine GC–FID monitoring of the volatile compounds in ten commercial thyme samples revealed that at least two different batches of one sample showed anomalies [1]. Particularly noticeable were the high contents of carvacrol, β-bisabolene, and thymoquinone as well as the low thymol and β-caryophyllene contents. Therefore, this sample should be investigated in more detail to clarify the causes. The occurrence of different chemotypes in thyme declares the chemical variability of the volatile compounds, in particular of thymol and carvacrol. However, the high content of thymoquinone could not be explained by this. It therefore appears probable that a contamination was responsible for the anomalies. Literature research showed that leaf samples from black cumin (*Nigella sativa*), oregano (*Origanum vulgare* and *Origanum onites*), rosemary (*Rosmarinus officinalis*), egoma (*Perilla frutescens*), ground ivy (*Glechoma hederaceae*), winter savory (*Satureja montana*), and capitate thyme (*Thymbra capitatus*) as well as seed from black cumin (*Nigella sativa*) came into focus as impurities due to the high content of thymoquinone and / or carvacrol. However, analyzing the volatile compounds by GC–FID, it was not possible to identify the contamination. Therefore, the polyphenols served as an additional feature for the authenticity of thyme. The HPLC–DAD profile of the contaminated samples was characterized by a high lithospermic acid A content and the occurrence of salvianolic acid B. With these two compounds it was possible to identify the contamination successfully as Origanum onites. It appears to be a deliberate falsification of thyme.

[1] G. Wittpahl et al., poster presentation 6th RAFA Prague 2013

Keywords: *thyme, authenticity, Origanum onites, falsification*

B32
TRACING THE GEOGRAPHICAL ORIGIN OF WHEAT FLOUR IN BREADS -PART 1: EXTRACTION AND COMPARATIVE ANALYSIS OF WHEAT PROTEINS IN BREADS

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Food fraud is a serious problem; therefore, the determination of analytical methods for the discrimination of the geographical origin of foods is desirable. To date, methods such as stable isotope and trace element analyses have been widely used for determining the geographical origin of food raw materials. However, conducting these analyses for one of the raw materials in processed foods can sometimes be challenging because processed foods are modified by the cooking process and also contain multiple raw materials. Wheat contains four kinds of protein fractions according to their solubility: water-soluble albumins, salt-soluble globulins, alcohol-soluble gliadins as well as acid- and alkali-soluble glutenins. The wheat gliadins and the glutenins constitute approximately 75–85% of the total seed proteins, with glutenins constituting almost half [1]. Additionally, they are recognised as the major wheat storage proteins [2,3]. The gliadins and glutenins compose gluten, which affects the qualities of food products made from wheat, such as breads and noodles. Pretreatment for tracing the geographical origin of wheat flour in breads involved the extraction of albumin/globulin and glutenin fractions from wheat flour and breads and a comparison of the qualitative differences using one-dimensional (1D) sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE). The 1D SDS–PAGE pattern of the albumin/globulin fraction extracted from wheat flour was different from that extracted from breads. On the other hand, the electrophoretic patterns of the glutenin fractions have little differences among wheat flour and breads. These results suggest that the glutenin fractions are rarely affected by the cooking process of breads. The results of 1D SDS–PAGE additionally revealed that the albumin/globulin fractions extracted from some breads containing different kinds of auxiliary materials such as yeast, butter and milk had variable electrophoretic patterns depending on the kind of added auxiliary materials. In contrast, the gluten in fractions in some breads showed a similar electrophoretic pattern in spite of the kind of auxiliary materials used. These findings suggest that the glutenin fractions in breads are mainly composed of the proteins from wheat flour in breads and are not affected by the addition of auxiliary materials. Thus, we conclude that the analysis of glutenin fractions would be potentially useful for tracing the geographical origin of wheat flour in breads.

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[2] Belderok, B., Mesdag, J., and Donner, D. A. (2000). Bread-making quality of wheat: a century of breeding in Europe: Springer Science & Business Media.
[3] Abdel-Aal, E.-S., Salama, D., Hucl, P., Sosulski, F., and Cao, W. (1996). J. Agric. Food Chem., 44(8), 2117–2123.

Keywords: *wheat cultivar, bread, protein, geographical origin*

B33
TRACING THE GEOGRAPHICAL ORIGIN OF WHEAT FLOUR IN BREADS -PART 2: STABLE CARBON, NITROGEN AND OXYGEN ISOTOPE ANALYSIS OF WHEAT PROTEINS IN BREADS

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Accompanying the globalisation of the food trade is the adoption of labelling policies by an increasing number of countries for protected geographical origin indication. Thus, there is a need for developing various analytical methods to validate the authenticity of foods. Stable isotope and trace element analyses have been widely used to identify the cultivation areas of food materials. However, these studies mainly focused on food materials such as fresh fruits, cereal crops and meats because the effect of the cooking process and auxiliary materials in processed foods complicates the assessment. Notably, food fraud has occurred not only in fresh foods but also in processed foods. Although the stable isotopic composition is relatively less affected by the cooking process, there is a need for removing the effect of auxiliary materials. Therefore, we have focused on the specific protein fraction of the target raw materials when assessing the geographic origin of foods. As a first application, we targeted the protein fractions of wheat flour in breads. In our recent study, the glutenin fractions in some breads showed a similar electrophoretic pattern despite the presence of auxiliary materials such as yeast, butter and milk. Therefore, in this study, we utilised the stable isotope analysis of wheat proteins in bread samples for tracing the geographical origin of their wheat. There was no significant difference in the δ13C and δ18O values ($P < 0.05$) of the glutenin fraction in bread samples with or without auxiliary materials. Moreover, these values were positively correlated with those of wheat flour, suggesting that the δ13C and δ18O values of the glutenin fraction from breads reflected those of wheat flour. Thus, we determined these values in bread samples which were composed of wheat flour from Australia, Canada, the USA and Japan. The δ13C values of samples from Australia, Canada and the USA were greater than those of samples from Japan. Samples from Australia had the greatest δ18O values. Thus, stable isotope analysis of the glutenin fraction would be a potentially useful tool for determining the geographical origin of wheat flour in bread samples. Although further investigation is required, stable isotope analysis of the specific protein fraction of the target raw materials may potentially be employed as a novel method to discriminate the geographical origin of processed foods.

Keywords: *wheat cultivar, bread, protein, geographical origin, stable isotope analysis*

B34
AUTHENTICITY TESTING OF TODDY SAMPLES (PALM WINE) USING SULFATED ASH CONTENT

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Coconut toddy (palm wine) is an effervescence beverage tapped from young inflorescence of coconut tree (*Cocos nucifera*). The sap contains various nutrients mainly sugar (16%), though it undergoes natural fermentative changes when it is stored. Toddy is a traditional drink in Sri Lanka. The fermented toddy is distilled and aged in an indigenous tree species barrel of oak or halmilla (*Berrayacordifolia*), called arrack. In Sri Lanka artificial fermented liquor is made out from the fermentation of sugar solution.There have been several complaints with respect to adulterated toddy. Ash values used to find out quality, authenticity and purity of substances and these values are important quantitative standards. Sulfuric acid is added prior to heating to facilitate the destruction of organic matter and to fix certain metals as their sulfate salts to prevent volatilization. When sulfuric acid is used, the resulting material is known as sulfated ash. Hence this study is mainly focused to identify authenticity of toddy using the sulfated ash content. Genuine toddy samples were collected from different areas and artificial fermented liquor samples were made in the laboratory using sugar solution (16%) and inoculum of genuine toddy samples. Different ratios of genuine toddy and artificial fermented liquor mixtures were prepared. Sulfated ash content of genuine toddy samples, artificial fermented liquor samples and different ratios of genuine and artificial fermented liquor were determined by using muffle furnace. Linear regression was obtained with sulfated ash content vs different ratios of genuine toddy samples. Sulfated ash content of genuine toddy was varied from 0.45 to 0.50 g/100 ml. Further toxic elements and nutritional elements were determined by using ICP–MS.

Keywords: *genuine toddy, artificial fermented liquor, sulfated ash content, adulteration*

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B35
DETERMINATION OF 5-HYDROXYMETHYLFURFURAL AND SACCHARIDES IN MEAD USING LIQUID PHASE SEPARATION TECHNIQUES

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5-hydroxymethylfurfural (HMF) is heterocyclic aldehyde, which is presented in foods containing saccharides. This compound is formed from simple sugars (glucose and fructose), as product of acid catalyzed dehydration or Maillard reactions. Its occurrence and accumulation in honey depends on the type of honey, storage conditions, temperature and pH. The warming up of the honey during production of mead could cause the increase of the content of HMF in the final product. In this work, the determination of HMF together with saccharides was performed in 24 samples of meads obtained by different producers. Reversed-phase liquid chromatography and micellar electrokinetic chromatography coupled to spectrophotometric detector was employed for the analysis of HMF. The most important saccharides in meads, glucose, fructose and sucrose, were determined using hydrophilic interaction liquid chromatography with chemical bonded aminopropyl stationary phase and 80% acetonitrile as a mobile phase. The detection was performed using refractive index detector. Content of HMF and saccharides highly vary in dependence on technological process of meads. Their content was lower in honey wine, where other saccharides are not added after technological process. The additional sweetening or coloring of mead can lead to the increase of HMF and saccharides contents.

Keywords: *5-hydroxymethylfurfural, saccharides, mead, liquid chromatography, micellar electrokinetic chromatography*

B36
DETERMINATION OF BOVINE AND PORCINE PROCESSED ANIMAL PROTEINS IN FEED FOR FARMED ANIMALS BY ELISA: PROOF OF CONCEPT BASED ON PEPTIDOMIC APPROACH

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Within the European Union (EU), the use of processed animal proteins (PAPs) including meat and bone meals (MBMs) as feed material for farmed animals has been totally prohibited after the crisis of Bovine Spongiform Encephalopathy (BSE). However, the EU decided to lift the feed ban for non-ruminant PAPs use in fish feed in 2013. This modification of the legislation requires techniques able to determine the tissue and species origin of proteins present in feed for farmed animals. Indeed, even if the reintroduction of certain species in non-ruminant feed is accepted; the use of ruminant PAPs and intra-species recycling is not allowed. Since animal by-products are subjected to hard conditions during the rendering process (high temperatures/pressure and/or chemical treatment); thereby leading to either the denaturation or degradation of proteins. It is the reason why, a peptidomic approach has been choose to identify specific markers for animal proteins from pork and beef. Three bovine and four porcine peptides have been selected, synthesized and then, coupled to a carrier protein in order to obtain polyclonal antibodies. These antibodies have been characterized and used in an indirect competitive enzyme-linked immunosorbent assay (ELISA). After assay's optimizations in buffer, several experimental protocols for protein extraction from PAPs have been assessed. This original concept has been proved for one out three bovine peptides and for one out four porcine peptides. The detection limits will be determined using spiking samples; knowing that the presence of PAPs at 0.1% is considered as the limit of detection in the official method protocol.

Keywords: *PAP, MBM, ELISA, peptides, feed, screening*

Acknowledgement: *This work fits into the "Peptido-genomic" project (RF11/6243) and has received funding from Federal Public Service of Health, Food Chain Safety and Environment*

B37
DIFFERENTIATION OF MANUKA AND KANUKA HONEYS BY MASS SPECTROMETRY AND CHEMOMETRICS

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New Zealand manuka (*Leptosperum scoparium*) honey has high antibacterial activity and is marketed and traded as one of the most medically effective honeys. The authentication of manuka honey is of the great importance; it has been reported that much more manuka honey is sold on the market than is actually produced. Kanuka (*Kunzea ericodis*) honey is one of the major contaminant of manuka honey because both plants grow in New Zelanad and pollen from both plants can lead to blended honey. Pollen of these two plants is almost identical and indistinguishable by microscopic pollen analysis. However, kanuka honey has only weak antibacterial activity. Therefore, more advanced techniques are necessary for the differentiation of manuka from kanuka honey. Authentic manuka and kanuka honey samples were collected from hive sites in the North Island (Northland, Wairarapa, Wairoa, Hawkes Bay, East Coast, Taupo, and Waikato) of New Zealand and analysed by ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry (UPLC–QToF–MS) coupled to multivariate data analysis (MVA). Using untargeted metabolomics and principal component analysis, reliable discrimination was obtained between manuka and kanuka honeys, as well as between honeys sampled in different regions. Some of the metabolites that clearly discriminate the sample groups were tentatively identified through database searching using Progenesis MetaScope.

Keywords: *manuka, kanuka, authentication, mass spectrometry, chemometrics*

B38
APPLICATION OF TARGETED LC–MS/MS ANALYSIS FOR THE CONFIRMATION OF OREGANO ADULTERATION

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In recent month the attention of the public was drawn by yet another food fraud scandal – this time oregano adulteration. A set of commercially available oregano samples were previously analysed in our laboratory by mean of two screening methods i.e. FTIR and untargeted LC–QToF–MS. Results revealed that almost 25% of the samples tested possessed some form of bulking agent, the majorly in the form of myrtle and/or olive leaves. Even though the two mentioned analytical methods present a number of advantages, for the purpose of adulteration confirmation and its quantitation, utilization of targeted LC–MS/MS was explored to determine if a single, powerful, rapid testing method could be devised. A total of 13 markers for adulterants such as myrtle, olive leaves, sumac, hazelnut and phlomis, were selected during statistical analysis of untargeted LC–MS data. Subsequently, a confirmatory method was developed on a Waters Xevo TQ-S mass analyser coupled to Acquity UPLC I-Class system, to facilitate a fast and reliable detection of oregano samples adulteration at levels as low as 5%. The correlation of the data generated from the three testing methodologies (FTIR, LC–QToF, LC–MS/MS) as well as their suitability to become standard testing methods for routine control will be discussed.

Keywords: *oregano, adulteration, mass spectrometry*

B39
INSTANTANEOUS IDENTIFICATION OF MEAT, CHOCOLATE AND CHEESE PRODUCTS WITH RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY

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There has been a significant increase of the incidence rate of food fraud cases related to products from unknown and uncontrolled origin. Consequently, there is an urgent need for method capable of the instantaneous characterization of different food products. The recently developed Rapid Evaporative Ionization Mass Spectrometry (REIMS) has been used for the analysis of various animal and plant tissues and has shown to be capable of the near instantaneous identification of different tissue types based on their mass spectral lipid fingerprint. In this study, we aimed to test the feasibility of REIMS analysis for a number of different food products. The results successfully demonstrate that our method is capable of the identification of different cheese, chocolate and liver products. Food grade liver of eight different animal species (pork, calf, lamb, rabbit, goose, chicken, duck and turkey) were purchased from commercial retailers. Eight soft and hard cheese varieties and ten chocolate (containing 0–99% cocoa) products were also obtained from commercial source. All liver samples were analyzed using a modified monopolar REIMS electrode, while chocolate and cheese samples were analyzed using a surgical CO_2 laser. A 4 m long 1/8" outer diameter transfer line was used to capture and transfer the generated plume to the atmospheric interface of the Xevo G2-S mass spectrometer. Data was recorded in the mass/charge range 150–1,000 and subjected to multivariate analysis after spectral pre-processing. Multivariate models were tested using cross-validation and a separate test set for classification. The acquired spectra featured ions associated with fatty acids (incl. very long chain fatty acids) in the 150–500 *m/z* region, glycerophospholipids in the 600–900 region and triglycerides in the 900–1,000 region in all cases. The full spectral fingerprint was subjected to principal component analysis (PCA) in order to reduce the data dimensionality and remove noise, followed by a linear discriminant analysis (LDA) for classification. A clear separation of different cheese products could be observed on the 3 dimensional PCA plot, while cross-validation revealed 97–100% correct classification in case of liver and chocolate products. The amount of cocoa within the chocolate showed a linear correlation on the PCA plot, leading to the assumption, that REIMS could be capable to estimate the cocoa content of the chocolate. A separate test set was used to test this assumption and the percentage of the cocoa content could be recognized. We have shown that a REIMS based rapid food characterization approach is capable of the classification of different cheese, liver and chocolate products including the estimation of cocoa content within each chocolate type. REIMS technology represents a new milestone across many food safety and authenticity applications by providing a real-time, reliable, cost effective and simple method for the analysis of food products.

Keywords: *mass spectrometry, food fraud and authencity, rapid identification of food products, REIMS*

B40
HOW CAN THE SCIEX TRIPLETOF® SYSTEM BE USED TO ANALYZE THE AUTHENTICITY OF YOUR WINE?

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Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide range of molecular weight, such as pesticides, veterinary drugs, mycotoxins and other food residues and contaminants. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers shows limits. It can be only used for expected analytes. Non-target (unknown) data analysis to identify unexpected food contaminants and ingredients is not possible. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC–MS/MS run. Several red and white wines were analyzed by LC–MS/MS using the SCIEX TripleTOF® system operated in high resolution accurate mass MS and MS/MS mode. The acquired data were analyzed with XCMSplus, an offline version of the most cited metabolomics software in the world. This software is a powerful tool for statistical data processing. Additionally, the software is connected to a metabolite database which can be used to identify unknown found features.

Keywords: *accurate mass, time-of-flight, unknown, identification, authenticity*

B41
SCREENING AND IDENTIFICATION OF ADULTERANTS IN WEIGHT LOSS SUPPLEMENTS USING LIQUID CHROMATOGRAPHY COUPLED TO ACCURATE-MASS HIGH-RESOLUTION MASS SPECTROMETRY

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The dietary supplement market segment has been growing worldwide at 5% or more year over year for an extended period of time. One particularly popular category is weight-loss products. Unfortunately, these products are sometimes adulterated with synthetic weight-loss drugs that have anorectic or laxative effects (such as sibutramine and its analogs) and also with antidepressants to suppress side-effects of these drugs. Therefore, screening and identification of a wide range of adulterants are prerequisites for many supplement manufacturing and distributing companies to ensure consumer safety, comply with regulations and protect their brand. We developed a high-resolution/accurate-mass screening and identification method using a Q-Exactive Plus instrument, which provides a wide analytical range allowing for detection of both low (contamination) and also high (adulteration) levels, which can occur in real-world samples. A combination of full scan MS-data dependent MS/MS and all ion fragmentation (AIF) was employed to acquire data for both known (targeted) and unknown (non-targeted) compounds. Our data processing workflow incorporated an in-lab generated database of potential weight-loss supplement adulterants to match analyte retention time, precursor mass, isotopic pattern and up to ten exact-mass fragments. Additionally, the AIF option allowed for retrospective analysis of the data and search for compounds not included in the database. A simple dilute-and-shoot sample preparation was used and evaluated in the analysis of a wide range of weight-loss supplement sample types (capsules, tablets, oils, liquids, powders and gummies) that were purchased and analyzed together with over-spiked extracts to establish sample preparation and instrument detection/identification performance. Analyte detection and identification were evaluated in sample extracts fortified with a mixture of approximately 50 adulterants before the final dilution and filtration steps. Levels that ranged from 1 to 1,000 ppm were fortified onto 23 different samples purchased from online retailers and compared to solvent-based standards. Several criteria were employed in order to establish if conclusive identification could be made in each matrix at various levels. Progressing from the beginning to the end of the processing workflow; the m/z was extracted from the full MS with a 5 ppm window around a 30-second window at the expected retention time, an isotopic pattern match was made to the pseudomolecular ion and theoretical isotopes, finally, at least one MS/MS fragment from an in-house established database must be matched in the data dependent MS/MS or AIF sample spectra. Further work involving potential analogs to known weight loss adulterants was also investigated, establishing fragments common to larger groups of analytes, such as sibutramine, sertraline, or ephedrine alkaloids.

Keywords: *adulterants, weight-loss supplements, high-resolution mass spectrometry, screening, identification*

B42
NOVEL RAPID METHOD FOR THE DETERMINATION OF HONEY PHYSICOCHEMICAL PARAMETERS BY RAMAN SPECTROSCOPY TO ASSESS HONEY QUALITY

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Nowadays, according to the Codex Alimentarius, honey quality is evaluated by determining a number of physicochemical parameters, such as moisture, electrical conductivity, pH, free acidity, colour, sugar content and hydroxymethylfurfural content, diastase and invertase activities, among others, using official methods (AOAC, International Honey Commission). These methods are based on classical methodologies (gravimetric, volumetric, conductometry, refractometry and spectrometry) and chromatographic techniques. The compulsory use of several of these methods for assessing honey quality makes the analytical procedure for honey quality control costly, long and tedious; besides considerable amounts of solvents and reagents are consumed. To overcome these drawbacks, the use of vibrational spectroscopies combined with chemometrics is proposed in order to quantitatively determine such physicochemical parameters. Vibrational spectroscopic techniques allow to obtain rapid on-line non destructive information without performing any special sample preparation, and moreover to determine various physicochemical parameters in a single run. NIR, MIR and Raman spectroscopies coupled to chemometric techniques have been applied to different aspects of the analysis of honeys [1], allowing the quantitative determination of several physicochemical parameters for both the control quality [2,3], as fraud detection [4,5]. The objective of the present work is to develop a novel rapid method based on Raman spectroscopy and chemometrics for the determination of the honey physicochemical parameters. With this purpose, Raman experimental conditions, such as sample temperature, laser power, sample position on the Z axis, resolution and number of scans, for the analysis of honeys were optimised. Then the physicochemical data, obtained by official methods, and Raman spectral data of 313 Argentinean honeys were analysed by multivariate data analysis. Validated Partial Least Squares regression models were achieved to determine with acceptable precisions, honey physicochemical parameters, i.e. the contents of fructose, glucose, reducing sugars, sacarose, turanose, maltose and erlose, fructose/glucose ratio, free acidity, color, moisture, and electrical conductivity, by just recording the Raman spectra of the honeys.

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Keywords: *honey, physicochemical parameters, Raman spectroscopy, chemometrics, partial least squares*

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B43
DIFFERENTIATION BETWEEN FRESH AND THAWED MEATS

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Freezing of meat is one of the most widely used means how to prolong it´s shelf-life, although there are other technological reasons, why to use this method, such as elimination of parasites when final meat products do not undergo heat treatment. Ice crystals that form during the process of freezing have profound influence on the texture of meat. Even on cellular level, where membranes of intracellular organelles, as well as whole cells, are damaged. Organelle content is then released into extracellular space in the form of exudate, which leads not only to changes of the meat texture, but to decrease of the meat quality also, thanks to the loss of sensory and nutritive important substances. Therefore, question arises how to differentiate between defrosted and chilled only meat, so that final consumer is not deceived, as defrosted meat can be otherwise sold as fresh (chilled) meat instead. There are several methods, which can determine freezing process. In general, they can be divided between two main categories, enzyme and non-enzyme. The most widely used non-enzyme methods are microscopy, sensory evaluation, infrared spectroscopy and DNA methods. Enzyme methods are based on detection of mitochondrial enzymes, as they are released only when cellular membrane is damaged. Older enzyme methods include HADH and API-ZYMTM testing set. The former detects β hydroxyacyl-CoA-dehydrogenase enzyme, the later detects 19 enzymes. Not all of them are, however, of mitochondrial origin. The aim of this study was to verify usability of new methods, which use determination of citrate synthase and aconitase for differentiation between frozen and chilled meat. Citrate synthase is determinated using 5-thio-2-nitrobenzoic acid, which is created when 5,5´-dithiobis(2-nitrobenzoic acid) and acetyl coenzyme A react. It´s concentration is then measured using 412 nm spectrophotometry. Aconitase activity is determined using 340 nm spectrophotometry based on concentration of NADPH. Both methods were tested by two series of trials on four types of meat (beef, pork, chicken and salmon meat). The first of the series showed, that method which uses citrate synthase has better distinguishing properties compared to the method, which uses aconitase. Only the citrate synthase method was therefore used for the second set of tests. Both series showed that citrate synthase method can be used to distinguish frozen and chilled meat on high level of significance.

Keywords: *citrate synthase, aconitase, frozen, unfrozen, meat*

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B44
GEOGRAPHICAL CHARACTERIZATION OF ARGENTINEAN HONEYS BY RAMAN SPECTROSCOPY

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Argentina is a major producer of quality honey; 90% of the honey produced in Argentina is acquired by the United States and the European Union. The globalization of the world market makes the authentication and characterization of botanical and geographical origins of honey an important issue. The use of quality schemes has a long history in the marketing of agricultural products in Europe (Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) label), protecting the identity and quality of a product from certain region, in order to prevent fraud and illicit practices, and give it an added-value. Argentina also has a legal framework of PDO and PGI for agricultural food products. Quality labels are linked to the characteristics of the production systems, geographical origin, and cultural and historical practices. In particular, for honeys, sensorial, pollen and physicochemical characteristics depend largely on the botanical and geograph ical origin, and reflect regional aspects of beekeeping management. Honey is traditionally characterized by performing the analysis of physicochemical parameters, melissopalynological analysis and sensory analysis; which have a number of disadvantages. The official methods used to determine honey physicochemical parameters are tedious, time-consuming, and use considerable amounts of solvents and reagents. Pollen analysis requires skilled personnel with extensive experience in the field. Similarly, for honey tastings, a trained and experienced taste panel is needed, and with the additional disadvantage of not being possible to taste more than a small number of honeys in each tasting session. To overcome these drawbacks, vibrational spectroscopies (IR, Raman) are considered powerful alternatives due to their simplicity, speed, cost-effective and non-destructive character. The use of vibrational spectroscopic techniques along with chemometrics is one of the strategies proposed for discrimination of food origin (1). MIR, NIR and Raman spectroscopy and chemometrics have been used to assess the botanical (2-3) and geographical (4-5) origin of honeys. The objective of the present work was to develop analytical tools for the characterization of Argentinean honeys. With this purpose, Raman experimental conditions, such as sample temperature, laser power, sample position on the Z axis, resolution and scan number, for the analysis of honeys were optimised. Argentinean honeys from three different production regions were analysed by Raman spectroscopy and pattern recognition techniques to develop classification models to distinguish honeys according to their geographical origin.

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Keywords: *honey, Raman spectroscopy, chemometrics, pattern recognition, geographical origin*

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B45
NEW AND RAPID METHODS FOR THE IDENTIFICATION OF TOBACCO BLENDS AND ENDANGERED SPECIES IN SEIZED FORENSIC SAMPLES

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The European Union (EU) customs laboratories require methods to address tobacco smuggling and illegal trade in endangered animal and plant species. This demand has driven the development of new and rapid DNA-based methods within the DECATHLON project. With respect to the issue of tobacco smuggling, DNA profiling methods that make use of next-generation sequencing (NGS) technology are being developed that can be applied by the customs laboratories to track and trace individual batches of tobacco that have entered the EU. Currently, it is hard to identify the origin of counterfeit cigarettes and/or to determine whether two or more seizures of smuggled cigarettes match. DNA profiling methods will allow for the identification of batches of tobacco that can be applied if no knowledge is available as to the cultivars present in the batch or blend. The methods should be robust and allow to generate a reproducible profile for a specific batch of tobacco. Different profiles should be generated for different batches of tobacco, that may comprise a different set of tobacco cultivars, or, if feasible, a similar set of tobacco cultivars, but in different ratio(s). An overview of the methods and strategies for profiling tobacco batches will be presented. With respect to the issue of illegal trade in endangered species (CITES), the European customs laboratories require methods that can broadly identify any plant and animal species present in any sample under investigation. In the EU, enforcement is mainly focused at the borders, where illegally imported products, plants or animals will be seized by Customs and CITES Authorities. Usually, visual identification became more difficult when there is only a part of an animal or plant and only experts or laboratory analyses are needed to conclude which species is involved. The most difficult category is processed plant or animal parts that are pulverized and have become an ingredient of food supplements or traditional medicines. Such seized forensic samples often contain highly degraded DNA. In those cases, DNA barcoding and Sanger sequencing is typically performed which make use of short DNA sequences from a standardized region of the genome as a molecular diagnostic in species iden tification. A disadvantage is that this approach is not applicable to products containing more than one species. DNA barcoding combined with NGS, commonly referred to as DNA metabarcoding, promises to overcome the analysis problem of multiple ingredients. Within the DECATHLON project, DNA metabarcoding methods are being developed that will allow simultaneous identification of endangered species in processed forensic samples. Part of the development is to select suitable DNA extraction protocols, identify informative full-length and mini-barcode markers, and to optimize NGS protocols. The prospects and limitations of DNA metabarcoding for the detection and identification of endangered species identification will be presented.

Keywords: *tobacco, endangered species, DNA-based methods, NGS, metabarcoding*

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B46
DIFFERENTIATION OF GEOGRAPHICAL ORIGIN FOR CABERNET SAUVIGNON WINES WITH UHPLC–QTOF/MS COMBINED WITH CHEMOMETRIC ANALYSIS

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Food adulteration and mislabeling are often found in the market, particularly for the food with high commercial values. Such issues may pose potential risk and trust crisis to the consumers. Conventional analytical methods and the anti-counterfeit labeling techniques are insufficient to identify the product authenticity and determine the point of origin. Metabolic profiling based on the feature patterns of the small molecules in food is becoming of intensive interest for food authenticity and source tracing analysis. UHPLC hyphenated with QTOF/MS is of the key metabolic profiling platform to obtain the full picture of the less volatile small molecules in samples. Chemometric analysis allows further mining the data to specific feature patterns which can be used as criteria for classification. Here we are aiming at building suitable models for determination of the geographical origin of wines using UHPLC–QTOF/MS profiling followed with chemometric analysis. The reference samples of Cabernet Sauvignon wines were collected from three main regions of China and two wineries of USA. The samples were centrifuged and the resulting supernatants were analyzed directly by UHPLC–QTOF/MS. The raw data were mined using 'Molecular-Feature-Extraction (MFE)' algorithm, then imported into chemometric software, MassHunter Profile Professional (MPP) for data filtration, statistical analysis, clustering, model building and prediction. Data mining using MFE found that each wine sample contains thousands of small molecular features. Filtration through MPP according to the features occurrence frequency and statistical analysis narrowed down the features significantly. Principle component analysis using the derived features demonstrated that the samples could be well classified based on the geographical origins. These features were then applied for model building using partial least squares discriminant analysis (PLS–DA), back-propagation artificial neural network (BP–ANN), and Naive Bayes model (NBM) algorithms. The models were evaluated by comparing the discriminant accuracy and confidence. It was found that NBM model was suitable for differentiating the geographical origins of the wines from three regions of China and two wineries of USA, and BP–ANN model was better for region classification of the wines from three main regions of China. The developed models were further validated by testing additional refe rence samples with an overall accuracy of 86.7%. It suggests that the metabolic profiling approach by combing UHPLC–QTOF/MS with chemometric analysis can open a new way for geographical origin traceability of grape wines.

Keywords: *wine geographical origin, UHPLC–QTOF/MS, chemometric analysis, model building and prediction*

B47
A QUICK AND RELIABLE METHOD TO DETECT A-AMYLASE (DIASTASE) AND THERMORESISTANT A-AMYLASE IN HONEYS AS A MARKER FOR ADULTERATION

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The traditional methods to determine the α-amylase (diastase) activity in honey are the Schade and Phadebas® assays. Due to numerous drawbacks of these methods such as enzyme specificity, slow manual preparation and the difficulty in obtaining standardized starch, we developed a new method to determine the α-amylase activity in honey that uses the most specific α-amylase substrate (5E-pNP-G7) and easily can be performed in automatic analyzer systems. Moreover, two year ago, a widespread problem popped up with Chinese honeys sold to the bakery industry using it as a replacement for plain sugar. Dough rising was bad and the cake volume decreased after baking. Finally the reason was traced back to a thermoresistant α-amylase which survived the whole baking process. Upon special request we developed an advanced α-amylase method where the residual α-amylase activity was measured after employing a rigorous heat treatment to destroy all natural honey enzymes. For pure authentic honeys, the natural enzyme activity rapidly decreases to zero during heat treatment (100% destruction). For honeys containing thermoresistant α-amylase the activity decrease is much slower and residual activity is still measurable after rigorous heat treatment. As a result, the novel technique contributes to a significant improvement of the authenticity control of honey and shows a significant effect on the elimination of adulterated honeys before entry into the market, thus strengthening the fair honey trade and the consumers' trust in natural high-quality products

Keywords: *honey, adulteration marker, thermoresistant α-amylase, diastase, nitrophenol*

B48
NEW USE OF NEAR INFRARED MICROSCOPY FOR THE DETERMINATION OF THE TAXONOMIC ORIGIN OF SOFT TISSUES FROM PROCESSED ANIMAL PROTEINS IN FEED

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The use of animal by-products in feed depends on their nature defined by the type of tissue or body parts and the species of origin. Currently, the detection of unauthorised processed animal proteins (PAPs) is based on light microscopy and PCR methods. Light microscopy identifies structures on the basis of their morphology and enables identification of particles (such as bones, cartilages, muscle fibres,…) while PCR is able to detect and identify the presence of specific animal DNA in feed. Nevertheless, for some scenarios, even combined, these methods do not succeed in determining the taxonomic origin of the PAPs. A typical example is that of an aquafeed containing authorised porcine PAP together with dairy products: the analysis will conclude of the potential presence of ruminant PAP. Therefore, there is a need for developing methods allowing a taxonomic characterisation of visual structures such as bones fragments and muscle fibres. For the characterisation of bones, NIRM has yet demonstrated its potential. However the limitation of NIRM is when the presence of bones is reduced or absent. This study investigated the potential of NIRM for the determination of the taxonomic origin of muscle fibres. The NIRM was experimented on 2 porcine PAPs vs. 6 ruminant PAPs and 7 fishmeals all of industrial origin. Results showed that NIRM allows differentiating muscle fibres from different taxonomic origins: fish, ruminant and pig. In addition to this taxonomic classification, results also reveal differences inside taxonomic clusters of PAPs (e.g among different ruminant PAPs and porcine PAPs). The results obtained on this type of meals are promising and offer new perspectives. Tests on adulterated feeds need to be performed by NIRM prior to validation.

Keywords: *near infrared microscopy, muscles, feed safety, processed animal proteins*,

B49
BOVINE BLOOD BIOMARKERS AS A WAY OF PROCESSED ANIMAL PROTEINS DETECTION IN FEEDINGSTUFFS

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The prohibition of using animal by-products in feedingstuffs depends on two factors: their nature defined by the tissue/cell type and the species of origin, and on their destination (pets, fur animals or other farmed animals). Proteomics is particularly well-suited to the purpose of PAPs detection as it is a tissue and species-specific method. The aim of this study was the identification and the selection of specific peptide biomarkers using tandem mass spectrometry for the detection of bovine blood products and blood meals in animal feed. Twenty-nine samples of blood meals and blood products (plasma or haemoglobin powder) of porcine, poultry and bovine origin as well as three milk products and two fish meals were analysed using a Q–TOF mass spectrometer. Vegetal feed samples adulterated with 1% or 10% of bovine plasma powder, haemoglobin powder or blood meal were also analysed to evaluate the applicability of the method. Four proteins of interest were highlighted: Alpha-2-macroglobulin, apolipoprotein A-1, serotransferrin and haemoglobin (α and β chains). From these proteins, sixteen peptides were identified as potential bovine blood biomarkers in feedingstuffs. Nine of them could be used for the detection of plasma powder and seven of them for haemoglobin powder or blood meal. The evaluation of these peptides by a search against NCBInr database revealed that some of them could also be used to detect other ruminant bloods such as ovine or caprine ones. These preliminary results are promising. Efforts are now focused to improve the protocol in order to increase the sensitivity of the method as regards the selected proteins.

Keywords: *tandem mass spectrometry, blood, biomarkers, processed animal proteins, feed safety*

B50
PROFILING OF TYPICAL UNIFLORAL HONEYS FROM SARDINIA (ITALY) BY MEANS OF PHYSICAL-CHEMICAL DETERMINATIONS, ANTIOXIDANT ASSAYS AND MULTIVARIATE ANALYSIS

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Honey is one of the most renowned natural foods and its peculiarities are strictly related to its origin. Consumers choose the type of honey to purchase on the basis of its organoleptic qualities, the alleged healthy properties and the declared botanical and/or geographical source. Therefore, the definition of the origin of honey is extremely important to value typical productions and to prevent frauds. For many years, melissopalynology has been the only way to the definition of the botanical origin of honey. Unfortunately, this kind of approach is not applicable to those honeys whose pollen is underrepresented or to filtered honey. For this reason, there is a growing interest in the development of new analytical strategies aimed to the authentication of honey origin. Beekeeping represents an important economic resource for Sardinia (Italy) and the honey amount produced in this area represents about the 11% of that of the whole country. In this study, we focused our attention on the four main unifloral honeys produced in the island: strawberry-tree (*Arbutus Unedo*), asphodel (*Asphodelus microcarpus*), thistle (*Galactites tormentosa*) and eucalyptus (*Eucalyptus camaldulensis*). The main goal of this work was the classification of the most typical honeys of Sardinia by means of simple and rapid physical-chemical determinations and multivariate analysis. Eight parameters (pH, free acidity, electrical conductivity, color, total content of polyphenols, antioxidant activity, antiradical activity and free fluoride content) were determined on a number of honey samples obtained from various parts of Sardinia. On a later stage, principal component analysis (PCA) and linear discriminant analysis (LDA) were applied to the resulting data set in order to differentiate among the botanical origins.

Keywords: *honey, Sardinia, multivariate analysis, Strawberry-tree*

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B51
USING THE ROUTINE SEPARATION DIMENSION AND IDENTIFICATION CRITERIA OF UPLC ION MOBILITY TO ENHANCE SPECIFICITY IN PROFILING COMPLEX SAMPLES

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The combined peak capacity of UPLC/ion mobility and collision cross section measurements (CCS) can be used to produce routine unequivocal identification of marker flavonoid isomers in complex mixtures such as herbal tea products (functional foods)/ phytomedicines). The genus Passiflora comprises of approximately 450 species, but only a few are commercially exploited. Several Passiflora (Passifloraceae) species are utilized as phytomedicines (sedative/tranquillising), the species contain flavonoids, mainly C-glycosylflavones (apigenin and luteolin derivatives; frequently occurring as isomers). Flavonoids are one of the largest and most wide spread classes of compounds and possess diverse pharmacological and biological properties.
Collision cross sections (CCS), accurate mass, fragment ions and retention time have been used to profile the hydroethanolic extracts of P. incarnata, P. alata, P. edulis and P. caerulea, grown in Brazil. This approach offers a unique selectivity in profiling complex mixtures. Results obtained clearly show the benefits of using the collisions cross section measurements and the combined peak capacity of UPLC with ion mobility. The enhanced peak capacity enabled more information to be extracted from fragmentation studies and the individual fragmentation spectra have been obtained for flavonoid isomers which are co-eluting. From the extracts characteristic assignment for 6-C and 8-C flavonoid glycosides isomers (vitexin and isovitexin) (orientin and isoorientin) were obtained.
Collision cross section measurements were obtained for the marker flavonoid standards, and this information was used to create a scientific library incorporating the expected CCS values. The four Passiflora extracts were analysed and routinely screened against the flavonoid CCS library, to determine the presence/unequivocal identification of the 6-C and 8-C flavonoid glycosides isomers. CCS measurements for marker glycoside pairs (vitexin and isovitexin) 188.8 Å2/195.5Å2 have been determined. For (orientin and isoorientin) 187.7Å2/198.1Å2 were obtained. This proved that it is possible to distinguish the marker isomer pairs for the extracts analysed using CCS measurements. When comparing the expected and the measured collision cross sections, the CCS measurement errors were typically <0.5%. In addition, it has been possible to acquire the cleaned up fragmentation spectra, which are mobility resolved from co-eluting components. In the case of isoorientin/orientin (which co-eluted chromatographically and had the same fragment ions), ion mobility resolution enabled unique fragment ion ratios to be observed. For the first time unique collision cross section measurements and corresponding isomer fragmentation spectra have been obtained.

Keywords: *collision cross section, ion mobility, enhanced peak capacity, authentication profiling*

B52
REAL-TIME AUTHENTICATION OF WHISKEYS USING DART–QDA ANALYSIS

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Whiskey labeling and branding is highly regulated to protect distillers and consumers. Development of analytical tools to quickly and easily authenticate whiskeys is therefore important to protect consumers and distillers alike. Direct Analysis in Real Time (DART) is a highly useful ambient ionization technique that benefits from the need for little to no sample preparation and no chromatography, allowing the user to directly analyze a sample within minutes. In this work, DART has been coupled with simple mass detection (QDa) to provide a tool for rapid identification of whiskey samples. Twelve different brands of whiskey were analyzed, including samples of bourbons (Kentucky and Tennessee), Irish whiskey, blended Scotch, and single malt Scotch. A DART-QDa method was established to provide the most unique mass spectra for each sample. The data obtained for all twelve whiskeys analyzed were used to construct a PCA and LDA based statistical model using a prototype model building software. The statistical model generated was used to make identifications of unknown whiskey samples as the mass spectra were being acquired or by a raw data file provided. Using the model created, unknown samples of each of the whiskeys were successfully identified in real time with greater than 97% confidence. Amongst all the samples, the five bourbons analyzed were most similar when statistically modeled. Despite their similarity, they were successfully identified by brand in the twelve whiskey model. The current study demonstrates the utility of DART–QDa in the authentication of whiskeys. This technology could be used to rapidly screen bottles of whiskeys to determine the need for further analysis of suspect samples. The data also implies usefulness in applications in the distillery to monitor the quality of production and blending of whiskeys.

Keywords: *DART, QDa, authenticity, whiskey*

B53
USING CAVITY RING-DOWN SPECTROSCOPY FOR THE DETECTION OF FOOD FRAUD

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In 1988 a new spectroscopic absorption measurement technique called Cavity Ring-Down Spectroscopy (CRDS) was introduced by O'Keefe and Deacon. Due to the very high sensitivity the CRDS allows the determination of stable isotope. Contrary to the currently common spectroscopic methods, like Isotope Ratio Mass Spectroscopy (IRMS), it is a robust and cost-effective alternative. The GfL – Gesellschaft für Lebensmittel-Forschung mbH uses this type of stable isotope analysis to verify the authenticity of foods especially for fruit juice. The used CRDS analyzer (Picarro B2221-i) coupled with the Combustion Module allows the simultaneous determination of $\delta^{13}C$ and $\delta^{2}H$ from carbon dioxide and water resulting from the combustion of the sample. The Cavity Ring-Down Spectroscopy uses an optical resonator consisting of two highly reflective mirrors in which the light of a laser is reflected back and forth. The decay rate of the laser light with and without analyte is measured against the time to determine its related concentration. On the one hand the determination of the cavity ring-down time makes the measurement independent of fluctuations in intensity of the light source, on the other hand effective absorption lengths up to 20 km can be achieved. This leads to a very high sensitivity of the cavity ring-down spectroscopy comparable to other direct absorption spectroscopy methods. First results show that a significant differentiation of cane and beet sugar is not only possible by the measurement of $\delta^{13}C$ but also by the determination of $\delta^{2}H$. Furthermore the comparability of CRDS and classical IRMS determination could be shown by the measurement of the $^{12}C/^{13}C$ ratio of the sugar fraction in fruit juice according to ENV:12140:1996. The reproducibility (R) of the method is given by 0.6‰ and is clearly complied with the CRDS analysis.

Keywords: *CRDS, fruit juice, isotopic analysis, authenticity*

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B54
DETERMINATION OF VITAMIN A AND VITAMIN E IN INFANT FORMULA

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Infant formula contains nutritionally beneficial levels of vitamins A and E. These vitamins play an important role in infant health and development. Therefore, monitoring vitamin A and E levels in infant formula is a crucial part of the quality control program for infant formula manufacturers. The analysis of vitamins A and E in infant formula is challenging due to the nature of the matrix, the hydrophobicity of the compounds, and the number of vitamin A and E isomers present in a sample. In particular, resolution of the early eluting vitamin A palmitate isomers requires optimal HPLC conditions and high performance HPLC media. The AOAC official method 2012.10 outlines a normal-phase HPLC procedure utilizing both ultraviolet and fluorescence detection. Prior to HPLC analysis the reconstituted formula undergoes liquid-liquid extraction (LLE). This poster demonstrates the AOAC official method for the analysis of vitamin A and E in infant formula using optimized mobile phase conditions on a Luna® NH2 HPLC column. The method provides adequate separation of all vitamin A and E isomers with an analysis time of less than 12 minutes.

Keywords: *vitamins, food quality, infant formula, HPLC*

B55
RECOGNITION OF VEGETABLE OILS ADULTERATION AND OXIDATIVE CHANGES IN THERMALLY STRESSED FATS AND OILS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

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This work was focused on the authentication of oils and studies on thermal stability of fats and oils using non-destructive methods, this study was aimed at the evaluation of the potential of attenuated total reflectance – mid – infrared ATR–FTIR) spectroscopy combined with multivariate data analysis for the adulteration and qualitative changes recognition. Adulteration of edible oils has been encountered for many years. There are many adulteration ways, for example, high priced oils adulterated with lower priced oil, edible oil adulterated with non-edible oil and qualified vegetable oils adulterated with waste cooking oil. Within this study based on calibration models created for various oils satisfactory detection of adulteration was demonstrated. In addition to the edible oils adulteration, ATR–FTIR method was also used for the thermal stability study of selected edible oils and lard heated at elevated temperature (180°C) for a time simulating the frying process. Massive oxidation, especially of polyenoic acids during thermal stress of oils and fats is occurring. Lipid oxidation leads to creation of series of intermediates, where the most important are hydroperoxides. Hydroperoxides are unstable and decompose into secondary products, especially carbonyl compounds, cyclic fatty acids, epoxy- acids, hydroxy- and oxidized dimers of fatty acids. There are also pyrolytic reactions during thermal stress, which lead to the formation of trans-unsaturated fatty acids and polymeric compounds. In the frame of realized experiments non-heated rapeseed oil, sunflower oil and lard and then heated samples for predetermined times (1, 2, 4, 8, 12, 18, 24 hrs) were measured. Increasing of secondary oxidation products and trans- double bonds, particularly for sunflower oil were observed. Other results prove a clear decrease of the number of cis- double bonds and a trend of growing amount of carbonyl compounds, for all samples.

Keywords: *ATR–FTIR, authentication, oils*

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B56
FOURIER TRANSFORM INFRARED SPECTROSCOPY COUPLED TO CHEMOMETRIC TOOLS APPLIED FOR THE AUTHENTICATION OF FLOURS MADE OF CEREALS AND PSEUDO CEREALS

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Cereals belong to the most important crops grown. The most significant portion of cereal grains is milled and marketed as flour. This is then used to produce bread, cakes, biscuits and many other commodities. Cereals are important source of carbohydrates, proteins, lipids, vitamins and minerals, some of them contain considerable amount of bioactive compounds (flavonoids, β-glucans). Appreciable is also content of ash and fiber associated with maintaining of physiological functions of the GIT and cardiovascular problems prevention. Beside mentioned positives, common cereals can cause serious health problems for people with celiac disease because of allergy to contained gluten. This is one of the reasons, which lead to develop rapid screening methods to exclude adulterated cereal products. Cereals and their less known subset pseudo cereals represent a basis of human nutrition. They have different representations over the world. The group of true cereals includes wheat, rye, triticale, barley, sorghum, oats, maize, millet and rice. Typically, this cereals are not suitable for celiac with exception of maize, millet and rice. Currently used alternatives such as buckwheat, amaranth and quinoa belong to the group of pseudo cereals. The reason of their growing popularity is the nutritional and biological value, the absence of gluten and the assumption of higher content of healthy components in compared with commonly consumed cereals. This study was focused on flours made of various crops. Mid infrared spectroscopy combined with multivariate data analysis has been used to measure the profile spectra with the aim to evaluate the applicability of attenuated total reflectance for characterization of various types of flours. The experiments were intended to find if the tested method is able to distinguish among cereals, pseudo cereals and alternative crops using statistical processing of measured data. Infrared spectra measured for seventeen selected samples were processed by multivariate data analysis software tool to demonstrate differences in cereals, pseudo cereals and alternative crops. The potential of applied methodology to characterize the different flours was proven. Also possibility to detect flour adulteration was demonstrated.

Keywords: *cereals, pseudo cereals, alternative crops, flour, ATR–FTIR*

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B57
METABOLOMIC FINGERPRINTING EMPLOYING HIGH RESOLUTION MASS SPECTROMETRY FOR AUTHENTICATION OF ORGANIC AND CONVENTIONAL TOMATOES

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The identity and authenticity of products are current topics in food and feed science. Modern approaches that could improve the expensive and time consuming methodologies, such as metabolomics are emerging in food authentication applications. Metabolomics is concerned with both target and non-target analysis of small molecules (<1500 Da). Two complementary approaches are used for metabolomic investigations: metabolic fingerprinting and metabolite profiling. Metabolite profiling focuses on the analysis of a group of metabolites. Food fingerprinting techniques do not deal with the identification of all metabolites, but with the recognition of patterns. The genetic background of agricultural commodities and various environmental or other external influences affect the fingerprint of food matrices dramatically. Within this study Direct Analysis in Real Time (DART) ion source coupled to high resolution Orbitrap Mass Spectrometer (DART–OrbitrapMS) was applied on a wide set of tomato (Solanum lycopersicum) samples grown in two different cropping systems, conventional and organic, in year 2012 and 2013 (altogether 61 samples from Italy and Denmark were analysed). Acquired data were treated using a multivariate analysis: Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS–DA). Sample groups clustering was evident in both obtained score plots. For example, the PCA of metabolomics fingerprints revealed clear differences between samples from Basilicata and Emilia-Romagna regions (Italy) as well as a difference between years of harvest. Danish samples were completely separated from other samples. Also partial differences between types of cultivation were observed from PLS–DA. However, the year of production had a more significant impact on the measured met abolomic fingerprints in comparison with the farming system. Metabolomics fingerprinting/profiling realized by DART–OrbitrapMS is very useful tool for authentication of raw materials.

Keywords: *authenticity, DART–MS, metabolomic fingerprinting/profiling, tomatoes*

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B58
POTENTIAL OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR JOINT SUPPLEMENTS AUTHENTICATION

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Glycosaminoglycans (GAGs) are linear biopolysaccharides constituting one of the main components of cartilage. Their structure is characterized by repeating different types of disaccharide units. Two of them, hyaluronic acid (HA) and chondroitin sulfate (CS), have been shown a positive impact as prevention and during the treatment of joint diseases that affect millions people over the world. These evidences lead to the development and production of medicines and joint supplements based on these compounds. With regards to a growing popularity of these products, effective laboratory control of their quality and authenticity has become an issue of high concern.

For this reason, it is also essential to develop a range of analytical methods enabling reliable determination of HA and CS. Besides of dominant electromigration and chromatographic methods coupled with variety of detection techniques, infrared spectroscopy (IR) represents a conceivable analytical strategy applicable for characterization of GAGs, its speed, accuracy and non-destructive nature are the main benefits. IR may provide structural information on GAGs molecule, their molecular weight and degree of hydratation.

This study was focused on evaluation of a potential of Fourier transform infrared spectroscopy (FT–IR) for joint supplements authentication. In our experiments were measured spectra of standards HA, chondroitin-4-sulfate (CSA) and chondroitin-6-sulfate (CSC) and then spectra of samples of 11 joint preparations purchased at Czech market, all in powderized form. Measurement was carried out by attenuated total reflection (ATR) technique and spectra were collected in wave number range of 400-4000 cm-1. All samples were measured in two parallel (five times). Characteristic absorption bands of functional groups were identified in the measured spectra of standards and samples. The obtained data were processed by statistical program TQ Analyst using two statistical methods – discriminant analysis and similarity match.

Discriminant analysis (PCA) showed a possibility of statistical resolution of measured profiles. Obtained results were comparable with declared compositions on the packaging of analysed products. However, a significant influence of various additives on acquired spectra, was encouneterd making authentication process rather complicated. It is also necessary to further optimize sample preparation procedure and laboratory experimental conditions to improve repeatability of measurements and also to test alternative statistical methods of multivariate data analysis to test more possibilities for profiles distinguishing. Similarity match was used for comparison selected joint preparations with standards.

Keywords: *infrared spectroscopy, hyaluronic acid, chondroitin sulfate, discriminant analysis*

Acknowledgement: *This work was realized within the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/21537)*

B59
CRITICAL ASSESSMENT OF SPME–GC–HRMS POTENTIAL IN WHISKY AUTHENTICATION

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Whisky is one of the most popular spirits in the word. Since the most famous whisky is originally from Scotland, the Scotch whisky is very often the subject of adulteration. For this reason both consumer protection agencies and producers require analytical laboratories to support their consumer and brand protection programs with effective brand authenticity analyses. Plenty of methods have been developed for quality and authenticity of whisky, e.g. UV/VIS spectroscopy for evaluation of compounds formed during the maturation process, near red spectroscopy or capillary electrophoresis. Furthermore, gas chromatography (GC) coupled to flame ionization detector or mass spectrometry (MS) has been applied for the quality assessment and in some studies for analysis of volatiles compounds also solid phase microextraction (SPME) coupled to GC–MS was used. To the best of our knowledge, SPME–GC hyphenated with high resolution mass spectrometry (HRMS) representing a po werful instrument for non-target analysis has never been used for the evaluation of whisky adulteration. Therefore, we developed a new approach for critical assessment of whisky based on GC–HRMS. During the development of this method, several analytical GC columns (both polar and non-polar stationary phases) and different type of inlets (splitless and split) were tested. With this new procedure, more than 140 authentic whisky samples differing in region of origin, maturation in various cask and age, provided by The Scotch Whisky Research Institute were analyzed. For the chemometric evaluation of the data, principal component analysis (PCA), partial least squares discriminant analysis (PLS–DA) and orthogonal PLS–DA were applied. Using these statistical methods, we achieved adequate distribution of samples according to the type of whisky and the region based on specific markers.

Keywords: *whisky, authenticity, adulteration, GC–HRMS*

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B60
BLACK PEPPER AUTHENTICITY TESTING BASED ON SPME–GC/(HR) TOFMS

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Black pepper is valuable commodity on worldwide market and may become a subject of fraud. Cases of adulteration of ground black pepper with cheaper plant materials or mislabeling have been encountered. Smart approaches for the testing of black pepper authenticity are needed to disclose such practices and protect consumers. One of conceivable authentication strategies is profiling of black pepper volatiles.
The evaluated set of samples in our study consisted of 16 ground black peppers and in addition to these samples, other materials derived from pepper (oleoresin, spent – residual material of oleoresin production, pepper peels), that can be under certain conditions used for adulteration, were delivered by spice trading company. Beside of these samples, twelve more pepper samples were collected in retail markets in the Czech Republic.
In this study, black pepper volatile profiles were obtained using head-space solid-phase microextraction coupled to gas chromatography - mass spectrometry. Time of flight mass analyzer equipped with automated deconvolution & peak find algorithm was used for primary data acquisition (TruTOF, LECO, USA). Using the Statistical Compare feature of the ChromaTof software by LECO, compounds were aligned in all off measured samples and after the normalization of their areas, the statistical analysis was performed in Simca software (Ulmetrics).
All the samples, both labeled as authentic pepper by our commercial partner and those from retail market grouped together using principal component analysis. Other 3 samples separated clearly. Identity of volatile compounds evaluated as important markers of these outliers were confirmed, using the high resolution TOF instrument (Pegasus HRTOF, LECO, USA). For one of "suspect" samples, organic solvents used for oleoresin isolation were the most decisive compounds. Other separated samples showed similar profiles of volatiles to those of pepper related materials mentioned above.

Keywords: *SPME–GC/(HR) TOFMS , black pepper, authenticity*

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B61
IDENTIFYING DIFFERENT TYPES OF COFFEE USING METABOLIC PATHWAY DRIVEN TARGETED METABOLOMICS AND METABOLITE IDENTIFICATION BY HIGH RESOLUTION MS/MS METABOLITE SPECTRAL LIBRARY SEARCH

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This presentation will highlighting a workflow for combined non-targeted and pathway driven targeted metabolomics making use of the same high resolution LC–QTOF-MS data set in a proof of concept study based on a coffee metabolomics example. Combined with a novel high resolution metabolomics MS/MS library this leads the way to seamlessly detect and identify potential biomarkers. The acquired LC–MS data was initially evaluated using an untargeted workflow. This untargeted approach pointed to some metabolites as being characteristic for particular coffee cultivars. From those, N-methylnicotinic acid was identified and a targeted screening list was automatically generated using compounds present in characteristic metabolic pathways (in this case nicotinic acid metabolism). The novel Compass PathwayScreener software tool readily allowed creating this target compound list from the metabolic pathway. It also enabled to quickly screen for the presence of the compounds in the same high resolution full scan data files used for the untargeted workflow. Compounds with significant changes between cultivars were tentatively identified taking into account accurate mass and isotopic pattern information. These identifications were further substantiated by comparison of fragment spectra to a new metabolomics relevant MS/MS Library containing several thousand manually curated MS/MS spectra. At least 5 different collision energies were used to generate MS/MS spectra for each metabolite. This provides flexibility for spectral match using QTOF instruments with different instrumental settings. Manually annotated fragment ion structures and/or formula for Library MS/MS spectra were found to be particularly useful for structural confirmation and to interpret the fragmentation pattern of matched metabolites.

Keywords: *coffee, authenticity, metabolomics, high resolution spectra library search, QTOF*

B62
OXYSTEROLS DISTRIBUTION IN THERMALLY PROCESSED MEAT

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Sterol oxidation products (SOPs), known as oxysterols, have been extensively studied. Their harmful health effect (cytotoxicity, mutagenicity, carcinogenicity, inflammation and the promotion of atherosclerosis, neurodegenerative diseases) is well known. Oxysterols can be formed endogenously both by enzymatic or non-enzymatic pathways, from sterols present in plasma and tissues. They can also be found at low concentration in many commonly consumed food (meat, fish, eggs and milk and their products). Processing, cooking and storage conditions clearly affect (increase) the oxysterol formation. Inadequate culinary processing of food can also lead to significant increase the degree of sterol oxidation. Temperature, time, free access of oxygen, potential antioxidant protection, but also moisture and pH are the most important factors influencing the oxysterol formation. Oxysterols can be formed in various processed food. But baked meat and minced meat products con tain sufficient amount of precursors and favorable condition for sterols oxidation. Baked meat probably represents one of the most important sources of oxysterols dietary intake. Therefore, it is necessary to identify and quantify oxysterol formation in food, mainly in thermally processed food. The aim of this work was the evaluation of oxysterols content and distribution in baked minced meat products (meatloaf). SPE method was used for oxysterol separation from lipid fraction of processed material. Identification and quantification of oxysterols was provided by GC–MS. The results should be able to show if these kinds of meal are the important sources of oxysterols in our diet. Conclusion can contribute to proposal recommendation for baking of meat.

Keywords: *oxysterol, meat, baking*

Acknowledgement: *Financial support from the specific university research (MSMT No 20/2015).*

B63
RAPID IMMUNOASSAY FOR RAW AND HEAT-TREATED BOVINE MILK PROTEINS IN THE MILK OF OTHER SPECIES AND SOURCES

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Monoclonal antibodies (Mabs) were raised against bovine κ-casein, an important milk protein on the surface of casein micelles. The corresponding epitopes and the dominant amino acids (AAs) were found with ultrahigh-density peptide microarrays by which the specificity towards other milk proteins of cows and other species and sources could be predicted. Of one of these Mabs, the corresponding 5 AAs-containing epitope was found to be similar in κ-casein from cows and buffalos and not present in other milk proteins of other species and sources and this was confirmed with the rapid ELISA. Due to the small epitope and the applied inhibition assay format, the ELISA also works with denatured proteins in heat-treated milk, which is a unique feature compared to other immunoassays. As the epitope is located on the glycomacropeptide part of κ-casein, the ELISA also works for the detection of bovine rennet whey (powder), a by-product of cheese production and the cheapest milk product, added to bovine milk and to the milk of other species and sources.

Keywords: *κ-casein, milk proteins, bovine rennet whey, ELISA, milk fraud*

B64
EXPLOITING THE MICROBIOME: A NEW APPROACH FOR DETERMINING THE PROVENANCE OF FOOD – CASE STUDY USING PACIFIC OYSTERS

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Currently our analytical methodology for provenance assignment is limited; stable isotopic techniques can provide indications of geographical origin for commodities like meat, but and are not usually effective for mixtures and can suffer from lack of spatial resolution. Traditional DNA techniques can identify species/varietal origin but cannot be used to infer geographical origin of species that may move during a lifecycle or production process. Microbial communities associated with foods are derived from the environment in which the food is produced and are influenced by production method and the nature of the food itself. Thus a food-associated microbial community contains signatures indicative of origin and production/handling as shown by Taylor et al. (2014) [1]. These communities are often highly complex, and only recently has it become possible to analyse them in depth using next generation sequencing approaches. We hypothesised that sequencing data from a taxonomic marker gene reflecting bacteria present in oyster gill tissue can be used to differentiate Pacific oyster samples according to production site and related conditions like culture substrate or origin of spat. In November 2013, 110 depurated oysters were collected directly from production sites. Bacterial 16S rRNA gene sequences were generated by pyrosequencing for oysters from a selection of 11 major production sites around the UK. After analysis of the sequence data using QIIME (Quantitative Insights Into Microbial Ecology) differences in bacterial communities between locations and substrate were identified using multivariate statistical methods. We found strong links between the microbiome and geographical origin and also between substrate (eg whether oysters were grown on trestles or on the sea bed) and the microbiome. The length of time oysters had been grown at the sampling location was also weakly linked to community composition although this correlation was less strong than with the sampling location itself, indicating that the oyster microbiome is influenced most strongly by the water in the locality. The sequence data allowed us to identify specific taxa such that the microbiome of a given sample can be described as a set of operational taxonomic units (OTUs) defined by sequence similarity. Thus, it is possible to define attributes of the microbiome that are linked to location. Further work is on-going to assess how temporal shifts affect the oyster microbiome in the different production sites and identify robust microbiome markers.

[1] Taylor MW, Tsai P, Anfang N, Ross HA & Goddard MR (2014) Pyrosequencing reveals regional differences in fruit-associated fungal communities. Environmental Microbiology 16: 2848-2858.

Keywords: *provenance, oyster, microbiome*

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BIOANALYTICAL METHODS FOR FOOD CONTROL

(C1 – C27)

C1
EXTENDED SHELF LIFE (ESL) MILK IN AUSTRIA − DAIRY PRODUCTS WITH EXCESSIVE HEAT LOAD?

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The recent trend towards a longer keeping ability of pasteurized milk, without the negative flavour change normally associated with ultra-high temperature (UHT) treatment, has resulted in the development of extended shelf life (ESL) milk and other ESL dairy products. However, heating causes a significant loss of organoleptic and nutritional quality (e.g., cooked flavour; vitamin destruction). Therefore, different Time-Temperature Integrators (TTIs) have been used to evaluate the heat load of ESL milk products (e.g., ß-lactoglobulin, hydroxymethylfurfural, lactulose, furosine). Surprisingly, no consistent legal definition considering threshold levels for such TTIs exists within the EU food law for ESL milk. The objective of this study was to investigate the actual heat load of liquid milk (n = 200) and whipping cream (n=58) samples at retail in Austria, either raw, pasteurized, ESL or UHT, respectively. Based on the existing IDF standards, improved RP-HPLC methods were firstly developed for the analysis of furosine and acid-soluble ß-Lg in liquid milk using a Symmetry 300 C18 column (3.5 µm, 2.1 × 150 mm) (Waters). The established RP-HPLC method enabled the separation of whey proteins within 21 minutes and was used for determination of acid-soluble ß-Lg. Furosine was analyzed by ion-pair chromatography RP-HPLC within 7 minutes. The optimized HPLC method for the analysis of ß-lactoglobulin was then successfully transferred to UHPLC equipped with an Acquity UPLC BEH300 C4 (1.7 µm, 2.1 × 100 mm) column. Referring to liquid milk, half of the analyzed samples designated as ESL milk showed acid-soluble ß-Lg contents lower than 1.800 mg/L milk, which had been proposed as threshold level in Austria. Most of these ESL milk samples with excessive heat-load had a surprisingly low amount of native, ß-lactoglobulin (< 500 mg/L) and a high furosine content (> 40 mg/100 g protein), which was almost comparable to that of UHT milk. As ESL milk has shown a dramatic increase in Austria recently, and has been widely accepted in many other European countries (e.g., Germany) in the meantime, the nutritional and organoleptic quality of this new category of liquid milk needs to be controlled in the future urgently. Concerning whipping cream, ß-Lg content was very low in all samples and was definitely not appropriate to differentiate between pasteurized/ESL/UHT cream samples. In contrast, furosine levels increased with higher heat load of cream in the logical order pasteurized < ESL << UHT, and allowed a significant discrimination of heat load of these products. Thus, furosine content of 70 mg/100 g protein was suggested as an upper heating limit for pasteurized cream, whereas 100 mg/100 g protein could be accepted as obligatory limit for tolerable heat load of ESL cream. In conclusion, acid-soluble ß-Lg is definitely most suitable for heat load evaluation of liquid milk, whereas furosine proved to be a reliable indicator to assess the heat load of whipping cream.

Keywords: *extended shelf life (ESL) milk, whipping cream, heat load, ß-lactoglobulin, furosine*

C2
INACTIVATION OF PROTEINASE INHIBITORS IN SOYBEAN USING DIFFERENT CHEMICAL TREATMENTS

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The demand for other protein sources than of animal origin is constantly increasing, leading to a growing need for protein products derived from plant sources. However, many of the present food or feed plant protein sources contain different proteinase inhibitors, limiting the nutritional value of the proteins. In particular seeds of legumes have a high incidence of potent proteinase inhibitors, which may limit the application of the otherwise attractive leguminous proteins or require further purification. The legume soybean is a significant source of protein for animal feeds and for food ingredients, especially for producing of meat and dairy analogues. The proteinase inhibitors predominantly present in soybean are the Kunitz soybean trypsin inhibitor (KSTI) and the Bowman-Birk inhibitor (BBI). Due to the presence of these, protein derived from soybeans may be digested less optimal. Hence, it is of high importance to determine possible methods to inactivate proteinase inhibitors. So far the industry applies a harsh heat treatment, which leads to the inactivation of most of KSTI present in the soybean, but also to the denaturation of other important proteins. Additionally, BBI is mostly resistant to this treatment. Consequently, different ways for a more specific and gentle inactivation of proteinase inhibitors are sought and for this purpose the mechanism behind the inactivation needs to be understood. This work proposes the possibility to conduct different chemical treatments to affect the activity of the proteinase inhibitors. We consider the content and stability of disulfide bridges in both BBI and KSTI to be a key target to control the activity. The effect on disulfide bridges has consequently been examined in a model system consisting of glutathione (GSH) and its oxidized form (GSSG) by using a non-denaturing buffer system in micellar electrokinetic capillary chromatography (MECC). The method has been optimized to quantify the thiol and disulfide bridges, and effects of using different buffer composition and pH have been examined. The method developed was applied for analyses of effects on inhibitor activity upon a range of chemical treatments with the purpose to elucidate inactivation mechanisms and conditions as well as identifying possible industrially applicable treatments. Significant reduction in inhibitor activity has been found due to the different chemical tr eatments of both BBI and KSTI, thus showing promising alternatives to the normally applied heat treatment. Changes at a conformational level of both BBI and KSTI due to different treatments were also followed by MECC and circular dichroism (CD).

Keywords: *proteinase inhibitor, disulfide bridges, soybean, KSTI, BBI*

Acknowledgement: *HITFOOD (The Danish National Advanced Technology Foundation)*

C3
ANALYSIS OF MICROBIAL VOLATILE ORGANIC COMPOUNDS EMITTED BY TRICHODERMA AGGRESSIVUM GROWING ON DIFFERENT SUBSTRATA

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Mould is one of the most common indoor air pollutants; their growth is very probable in a wet environment. Mould can cause several allergic reactions; furthermore their presence is harmful for numerous industrial processes. Moulds contribute to the degradation of food products and can cause environmental damages e.g. sick building syndrome. A very mould-sensitive process is the production of bisporic button mushroom (*Agaricus bisporus*), which is often infected by the green mould disease (*Trichoderma aggressivum*). During their growing period, moulds produce different microbial volatile organic compounds (MVOCs), which are released into the air. It has been shown that the composition and the amount of these emitted MVOCs change over time. Sampling of volatile organic compounds (VOCs) in air has been shown to be a viable method for monitoring of the environment. Detection of microbial volatile organic compounds of moulds is a useful tool for monitoring mushroom production. This way it is suited to identify and indicate the presence of moulds in the air of the cultivation site. Because the produced MVOC pattern is highly substrata-dependent, the identification of mould species is usually difficult. Our main aim was to investigate the effect of different substrata on emitted MVOCs pattern and to create databases having identification capabilities. Headspace-solid-phase microextraction (HS–SPME) coupled with gas chromatography-mass spectrometry (GC–MS) was used to analyse microbial volatile organic compounds of mushroom disease-related microorganisms. *Trichoderma aggressivum f. europaeum*, which is typically harmful in mushroom cultivation, was examined. PDA (potato-dextrose agar), MEA (malt-extract agar), CA (mushroom compost agar) and WA (water agar) was used as cultivation substrata in-vitro, moreover mushroom compost was also examined as real substrata (*in-situ*). Several different marker compounds were found on each media, which enabled us to examine T. aggressivum in timely manner. According to our results, substrata-dependent databases were successfully built. Despite of similarity among different databases, these databases are characteristically substrata-dependent. This phenomenon can be caused by the different carbon source. To sum up, the applied HS–SPME–GC–MS methodology proved to be a useful tool for monitoring the MVOCs emitted by *T. aggressivum*. Monitoring of volatile marker compounds from the air enabled us the early detection this harmful mould. In the case of the investigated five media, the identification of *T. aggressivum* can be certitude carried out.

Keywords: *HS–SPME–GC–MS, Trichoderma aggressivum, MVOCs, headspace sampling*

Acknowledgement: *KMR-12-1-2012-0189*

C4
OPTIMIZATION STUDIES OF OPTICAL BIOSENSORS FOR FOOD APPLICATIONS

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Optical, label-free biosensors are a powerful detection and analysis tool that has vast applications in biomedical research, healthcare, pharmaceuticals, environmental monitoring and the food industry. This is, amongst other, due to the extremely low limit of detection that can be achieved with the use of such sensors. However, the recognition of small analytes, such as the mycotoxins or antibiotics that are of interest to the food industry, poses significant challenges due to the small shifts it causes in the recorded signal. Here, we report the work that has been carried out towards the detection of food contaminants, such as ochratoxin A and aflatoxin M1 with the use of aptamer-based microring resonators and present solutions to circumvent this problem which are focused in two main directions. The first one, consists in the development and optimization of a complementary to the immobilized aptamer DNA strand that gets removed once the target analyte is re cognized, thus increasing the shift that accompanies the binding of the food contaminate to the probe alone. The second advancement deals with the deposition of the aptamers themselves with the use of the laser transfer forward technique (LIFT) onto silicon nitride surfaces that have been custom-modified to allow the immobilization of the former with high spatial resolution and high densities. A combination of both methods will allow the realization of the full potential of optic-based sensors while the conclusions reached can be directly applied to many different types of biosensors, especially for food applications.

Keywords: *aptamer, mycotoxin, biosensor*

C5
A NOVEL IMMUNOASSAY FORMAT FOR RAPID SCREENING OF MYCOTOXINS – HT-2 TOXIN AS AN EXAMPLE

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Mycotoxins are secondary metabolites of fungal species infecting food, feed and raw materials e.g. wheat, barley, rye and maize. Infection can occur in the fields but also during the storage and processing. Mycotoxins have unwanted health effects for humans and animals such as haemorrhage, vomiting and impaired immune function. The most relevant mycotoxins are regulated by the authorities. Rapid methods for the detection of the mycotoxin contamination are needed for the efficient screening of high number of samples with fewer resources than required by the sophisticated analytical methods. Easy-to-perform immunodiagnostic methods provide tools for in situ decision-making preceding the further confirmatory analysis. Recombinant antibodies offer advantages over the traditionally used poly- or monoclonal antibodies in immunoassays. Recombinant antibody fragments can be isolated in vitro from the antibody gene libraries displayed on bacteriophages and produced cost-efficiently in large scale in *E. coli* bacteria. Recombinant antibodies can also be further engineered to meet the requirements of a certain application e.g. by improving their affinity, specificity or stability. Here we demonstrate how recombinant antibodies enable a novel and very simple non-competitive immunoassay for HT-2 toxin which is a mycotoxin produced by Fusarium spp fungi. We have developed recombinant antibodies against HT-2 and T-2 toxins from phage display antibody library containing ca 108 different antibody clones. Antibody clone having 100% cross-reactivity for HT-2 and T-2 toxins was used as a primary antibody in the development of HT-2 toxin specific anti-immunocomplex antibody from the VTT naïve antibody library. The anti-immunocomplex antibody recognises the conformational change due to the binding of the HT-2 toxin to the primary antibody. We have developed two novel fast and simple assay methods for HT-2 toxin with improved specificity and sensitivity. In the ELISA- based sandwich -type of assay the sample containing HT-2 toxin can be added together with the immunocomplex antibody- enzyme fusion to the wells pre-coated by the primary anti-HT-2/T-2 toxin antibody. After one hour of incubation and a single washing step the substrate can be added and the response corresponding to the amount of HT-2 toxin in the sample can be detected in 30–60 minutes. In the homogenous assay format the recombinant antibodies are labelled with fluorescent labels. Labelled antibodies can be pre-dried to the assay vials, e.g. microtiter plate wells, and readout is ready in 5-10 minutes after the sample application. This novel immunoassay format can be used to detect the possible mycotoxin contamination in raw materials, during the processes and in the final products. The promising new assay format for HT-2 toxin enables a fast, direct, sensitive and specific detection of the HT-2 toxin in an easy way: add sample and measure.

Keywords: *mycotoxin, HT-2 toxin, recombinant antibody, non-competitive, fast immunoassay*

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C6
APPLICATION OF MACHINE-LEARNING METHODS TO RECOGNITION AND CLASSIFICATION OF FOODBORNE PATHOGENS ON THE BASIS OF ELASTIC LIGHT SCATTERING CHARACTERISTICS

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The majority of tools for foodborne pathogen recognition and classification (e.g., ELISA, PCR) are based on physiological or genetic properties of microorganisms. Currently, there is an enormous interest in devising label-free and reagentless methods that would operate utilizing the biophysical signatures of microbial samples without the need for labeling and reporting biochemistry. MALDI–TOF is a known recent example of such an approach. However, elastic light scattering (ELS) – one of the most fundamental optical processes whereby electromagnetic waves are forced to deviate from a straight trajectory by non-uniformities in the medium that they traverse – can be employed to provide a much less expansive, yet equally accurate implementation of the label-free phenotypic classification concept. The major difficulty posed by ELS-based instrumentation lies in deciphering the highly complicated ELS patterns formed by bacterial colonies irradiated with laser l ight. Even though the well-developed light-scattering theory and accompanying computational tools such as dipole-dipole approximation could be used for modeling and subsequent interpretation of the raw ELS signals, such a rigorous approach to the inverse-scattering problem remains extremely difficult and computationally expensive. The presented work demonstrates a robust and rapid methodology for pathogen recognition, taking advantage of machine -earning (ML) and computer-vision tools for classification of ELS patterns formed by interaction between laser light and colony morphotypes. The constructed ML classifiers allow for rapid recognition of scatter patterns produced by the colonies without the need to use any specific model of light scattering on biological material. The described classification algorithms do not operate on raw ELS patterns, but utilize complex moments that are calculated in the polar coordinate space of the patterns using complex polynomials. The pseudo -Zernike, Fourier-Mellin, or Chebyshev-Fourier moment invariants are subsequently used to train various ML classifiers in order to recognize the unknown samples. The results demonstrate the use of the method to classify colonies of *Listeria*, *E.coli*, and *Salmonella* with accuracy above 95%, indicating that the demonstrated technology can be implemented in automated devices for detection and classification of pathogenic bacteria. Owing to reproducible morphological differences in internal colony organization, the method can robustly classify the samples on the genus, species, or even serotype level. We also discuss limitation of the presented approach, namely the reliance on a priori selection of features. We will conclude by briefly introducing possible future solutions such as unsupervised feature learning, which provides a framework for dynamic construction of ELS-derived features describing bacterial scatter patterns without any assumptions regarding the quality and informa tion content of these features.

Keywords: *foodborne pathogens, pattern recognition, elastic light scatter, machine learning, food microbiology*

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C7
RELEVANCE OF THE VOLATILE METABOLOME IN ANIMAL TISSUES TO REVEAL POLLUTANT CONTAMINATION IN THE FOOD CHAIN

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Among the environmental contaminants possibly transferred to animal-derived food, brominated flame retardants (BFRs) deserve attention because of strong evidence of increasing contamination of the environment, wildlife and people. Some toxic BFRs are part of the critical emerging contaminants, as hexabromocyclododecane (HBCD). Diet is one of the major routes of human exposure to HBCD and consumers may be exposed through a single food intake at very high doses of HBCD. The only way to guarantee the food safety given the unpredictable isolated cases of high contamination is to propose frequent and large-scale controls, yet the current reference analytical methods are economically inconceivable. Alternative approaches based on research in food of livestock's exposure markers to pollutants can be proposed. Among all biological compounds assumed as potential markers, the volatile organic compounds (VOCs) seem to be particularly promising. In a recent review, Hakim et al. (2012) proposed that metabolic disorders can produce new VOCs or change the ratio between the VOCs produced usually by the body. In this regard, the volatile metabolome in liver of animals exposed to environmental pollutants has been shown to differ from that of unexposed animals (Berge et al., 2011). The study aims to assess the relevance of the volatile metabolome in animal tissues to reveal an exposure to HBCD. Two groups of laying hens were given during 18 weeks a contaminated feed containing 5 or 50 µg α-HBCD /kg. Two other animal groups were fed the feed containing 50 µg α-HBCD /kg feed during the 11 first weeks then the control feed during the last 7 weeks, or only the control feed throughout the experiment. The results highlighted the relevance of the SPME–GC–MS for studying the VOCs in animal tissues and for determining the markers of HBCD exposure. The volatile fingerprints in egg yolk and in liver allowed the different animal groups to be clearly discriminated and the volatile metabolome in livers enabled to highlight markers of HBCD exposure of which relevance was assessed in the light of the literature data. The analysis of these markers could enable to reveal systematically suspect samples on market and to guide further analyses to confirm and possibl y explain the contamination.

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[2] Berge et al. Use of volatile compound metabolic signatures in poultry liver to back-trace dietary exposure to rapidly metabolized xenobiotics. Environ. Sci. Technol. 45 (2011) 6584-6591.

Keywords: *volatile metabolome, animal tissues, food safety, hexabromocyclododecane*

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C8
FISH SPECIES IDENTIFICATION BY PCR USING PARVALBUMIN GENE INTRONS AS A PLATFORM

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Identification of fish species from a piece of meat in complex or processed food is gaining increased importance due to differences in price of various species as well as of different allergenic potential among various fish genera and species. PCR–based approaches represent modern analytical methods to reach this goal. In the presented study, second intron in protein coding region of fish parvalbumin gene was chosen as the most promising area for such PCR. Degenerate primers were designed in adjacent exons based on broad alignment of available fish cDNA parvabumin sequences. Amplicons of each studied species obtained in PCR started from these degenerate primers were mined from agarose gels of corresponding end point PCR. Upon sequence of this DNA fragment species–specific primers were designed. Each such pair of primers was tested in comparison of PCR of respective species compared to PCR on panel of other fish species [1]. Robustness of the method in routine employment was confirmed in collaborative study of four EU laboratories [2]. Set of primers specific for Black seabream (*Spondyliosoma cantharus*) served as a marker in this study.
In other fish species, satisfactory results are being obtained at the pilot stage of the research. To bring these promising results into common routine employment, proper discrimination of faint “parasitic” bands appearing in some of the negative species turns out to be the most urgent task. Though this problem is relevant only in rare cases of very similar electrophoretic mobility of the marker band and this parasitic one a battery of approaches can be employed to cope with this phenomenon. Gradient of T_m –based optimization appears to be approach of the first choice. Selection of another set of primers within the intron is the alternative solution. Also transformation of the PCR into Real Time format with the specific probe is the possible option.

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[2] Laknerova I., Zdenkova K., Purkrtova S., Piknova L., Vyroubalova S., Hanak P. (2014): Interlaboratory identification of black seabream (*Spondyliosoma cantharus*) as a model species on basis of PCR targeting the second intron of the parvalbumin gene, Journal of Food Quality 37: 429-436, ISSN 1745-4557

Keywords: *PCR, fish species identification, parvalbumin gene, collaborative study*

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C9
SIMULTANEOUS DETECTION OF THREE PESTICIDES BY A WHITE LIGHT INTERFERENCE SPECTROSCOPY SENSING SYSTEM

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Contamination of food and drinking water by pesticides can pose a serious threat to human health and has therefore to be continuously monitored. EU authorities have determined both the maximum residue limits (MRLs) and the recommended detection methods, usually liquid and gas chromatography combined with mass spectroscopy. These methods provide for the simultaneous determination of a high number of analytes in a single run, with high accuracy and reproducibility. However, the instrumentation required is mainly for laboratory use by highly experienced personnel. Thus it is expected that the availability of small size and easy to operate tools with multi-analyte capabilities would facilitate more systematic food analysis for detection of harmful substances. In the present work, we employed a sensing platform based on White Light Interference Spectroscopy (WLRS) for the simultaneous label-free immunochemical determination of three pesticides, namely chlorpyrifos, thiabendazole and imazalil, in food and drinking water samples. Determination is based on immobilization on spatially distinct areas of a single sensing surface of the respective analyte-protein conjugates. The sensing module is composed by a Si wafer with a 1000 nm-thick SiO_2 layer assembled on a docking station with a microfluidic cell to allow for continuous fluid delivery. Detection is based on illumination of the sensing surface with white light using a reflection probe consisting from a bundle of six fibers at the periphery of probe. As the light beam is reflected on the layers of the sensing element with the different refractive indexes, an interference spectrum is created, collected by the central fiber of the reflection probe and guided to a miniaturized spectro meter. The accumulation of biomolecules onto the sensing surface when mixtures of calibrators or samples with analyte-specific monoclonal antibodies are run over the sensing surface increases the biomolecular adlayer thickness leading to reflected interference spectrum shift. Appropriate signal processing of the reflected spectra obtained in the course of the immunoreaction allows for its monitoring in real-time. The interrogation of the areas corresponding to different analyte is achieved by moving the sensing surface with respect to reflection probe. Using this system, the three targeted pesticides could be determined in less than 30 min with a limit of detection of 40 ng/mL, that is well discriminated from the MRLs set for drinking water (100 ng/mL). The same sensing surface could be used for at least 20 times after regeneration reducing considerably the analysis cost. Due to the small size of the sensing area (3.5 mm^2), the relatively low cost and compact instrumentation and the ability for multi-analyte, label-free and real-time determinations, the proposed sensing system could provide a viable solution for on-site pesticide determinations.

Keywords: *pesticides, label-free detection, simultaneous determination, White Light Interference Spectroscopy*

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C10
EXPLORING NANOFLOW LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY FOR PESTICIDE TESTING IN FOOD

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Pesticide testing in food is based on the use of multiresidue methods based on a generic extraction procedure, like QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) [1], followed by analysis using GC–MS/MS and UHPLC–MS/MS. Usually, in LC–MS/MS, LC flow rates exceed 400 µL/min and combined with small-particle size columns provide excellent peak shape and results although at the expense of relatively high solvent consumption. The reduction of flow rates in electrospray detection yields to an increase in sensitivity, which can be used for increasing the ruggedness of methods by means of, for instance the dilution of the sample extracts, thus minimizing matrix effects. Recently, the use of microflow-liquid chromatography tandem mass spectrometry has been proven to be an interesting alternative to standard analytical size approaches [2], provided the significant benefits in terms of sensitivity and matrix effect reduction. In this sense, the use of nanoflow liquid chromatography coupled to nanospray mass spectrometry detection has been restricted so far to selected bioanalytical applications (eg. proteomics), bearing in mind the difficulties associated to adapt such specialized approaches to routine applications. The relatively recent introduction of more robust and reproducible ultra-high pressure nanoflow LC instrumentation along with new column technology integrating nanoLC column and nano-ESI spray emitter in an easy-to-use plug-and-play fashion has made accessible such sophisticated approach to routine work, avoiding typical nanoLC issues such as leaks and minimizing other problems related to dead volumes/junctions. In this communication, the performance of nano-flow UHPLC combined with high-resolution mass spectrometry (using an orbital ion trap analyzer) has been evaluated for pesticide determination in food. Selected aspects such as retention time and peak area reproducibility, method s ensitivity, column tolerance and endurance to different matrices and matrix effects have been evaluated for a suite of over 60 multiclass pesticides. Limits of quantitation in the ng/Kg range were obtained with retention time RSD (%) < 0.3 % and peak area RSD (%) below 10 % in most cases.

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Keywords: *nanoflow UHPLC, pesticides, high resolution mass spectrometry*

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C11
MICROBIAL MICROARRAYS FOR THE DETECTION OF FOOD- AND WATER-BORNE PATHOGENS

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Food and water borne pathogens are potential risks for public health. The large number of microbial pathogens in a high number of samples require detection methods that allow multiplexing and highthroughput analysis. DNA microarray technology fulfills these requirements. They are tools for the simultaneous, sensitive and specific detection of pathogens in a relatively short time with the potential of automation. These features are significant advantages compared to classical culture-based pathogen analysis methods. High quality DNA microarrays require well-matched combinations of suitable surface functionalized substrates for capture probe immobilization, the most advanced array printing technology, incubation protocols and detection technologies. The SCIENION AG has developed a DNA microarray platform with tailored substrates and immobilization technologies for oligonucleotide probes. The non-contact sciFlexarrayer printing technology is applied to obtain planar microarrays of highest quality. As an example for miniaturized multiplexed DNA assay applications the results of an EU-funded study to develop an array based molecular tool for detection of microbial targets as bacteria, viruses and protozoa in fresh water samples are presented.

Keywords: *DNA microarrays, pathogen detection, water analysis, multiplexing*

C12
USE OF DIELECTRIC BARRIER DISCHARGE IONIZATION (DBDI) WITH LIQUID CHROMATOGRAPHY/ HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF MULTICLASS CONTAMINANTS IN FOOD

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Liquid chromatography-mass spectrometry (LC–MS) with electrospray ionization (ESI) is generally used for the analysis of relatively polar compounds. However, the analysis of nonpolar species is commonly undertaken by GC-MS, as they are not effectively ionized in the ESI source. With the aim of extending the applicability of LC-MS coupling towards a wider range of compounds with different physicochemical properties, alternative ionization sources have been proposed. The main alternative to ESI is so far atmospheric pressure chemical ionization (APCI) source. Another alternative that has been proposed is atmospheric pressure photoionization (APPI). Besides, several mass spectrometry vendors have proposed hybrid ionization techniques (eg. ESI/APCI). Recently, an ion source for LC-MS based on dielectric barrier discharge principle was reported by Hayen et al (Anal. Chem.. 81 (2009) 10239). Due to the different species generated in the plasma jet, the DBDI source offers the ability to generate not only positive but also negative ions, as various mechanisms including electron capture and proton transfer apply at the same time. The eventual combination of this ionization source coupled to a mass spectrometer featuring fast polarity switching (i.e. > 5–10 Hz) may provide a universal method covering a vast range of compounds with different physicochemical properties. In this communication, we have coupled this dielectric barrier discharge plasma jet with different LC-MS instruments from three manufacturers featuring atmospheric pressure interfaces with completely different geometry and conditions: AB Sciex (TurboIonSpray, QTRAP 4000) Agilent Technologies (orthogonal APCI source, Agilent TOF 6220) and Thermo Fisher Scientific (IonMax, Exactive Orbitrap). Selected examples on the application of the HPLC–DBDI–MS coupling are shown including the determination of multiclass lipids, trace analysis of contaminants of emerging concern such as ph armaceuticals in wastewater and pesticide determination in foodstuffs.

Keywords: *LC–MS, ionization, dielectric barrier discharge*

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C13
APPLICATION OF THE STANDARD ADDITION METHOD IN THE EROD ASSAY FOR QUANTITATIVE BIOMONITORING

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The EROD assay is one of the most widely used bioassays for biomonitoring of persistent organic pollutants in food, feed and environmental samples. Commonly dioxins and PCB contaminations in samples are quantified by a standard curve based on either 2,3,7,8-TCDD or beta-naphtoflavone. By using a standard curve approach for quantification of the sample, the unknown sample concentration is interpolated from the known concentration of the standard curve. This means the measuring properties of the standard curve should also apply to the measurement of the unknown sample to enable reliable quantification. However, in biomonitoring it is common sense that there are matrix effects present, which can significantly influence sample quantification. Therefore using a standard curve for quantification may neglect matrix effects present from diverse sample origins. To address these challenges in biomonitoring we investigated the applicability of the standard addition method for the quantification of dioxins by the EROD assay. The applied EROD assay was performed with the rat hepatoma cell line H4IIE in a 96-well format and 2,3,7,8-TCDD as standard substance. A diverse set of samples derived from food and feed stuff was analyzed. The standard addition method was applied in comparison to the commonly used standard curve approach and the results of both quantification methods were compared to each other. Additionally all samples were also quantified by HR–GC/MS for comparison. The results show a good correlation between both EROD based methods, the standard curve and standard addition method. In contrast the measurements obtained by HR–GC/MS generally resulted in lower toxic equivalents measured, presumably due to biological active contaminants only measured by the EROD assay. Additionally for the standard addition method; the concentration of the added standard substances need to be within a narrow range to warrant linearity for measurement. This is in contrast to the standard curve method where a broader concentration range is required for appropriate sigmoidal curve fitting.

Keywords: *dioxins, feed food control, EROD bioassay, biomonitoring, standard addition method*

C14
DEVELOPMENT OF APTAMERS FOR THE DETECTION OF TYPE A TRICOTHECENE MYCOTOXINS: USE OF CAPTURE SELEX

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In recent years the occurrence of fungal toxins within food and feed has become of increasing concern. In particular, toxins produced by fungal species that contaminate grain products has become a focus of attention as levels are increasing and cause potential health issues. Specifically, fungal toxins produced by the Fusarium species are becoming more common. This research study focusses on Type A trichothecenes: T-2 and HT-2 toxin, as so far these toxins have not been widely investigated. The development of rapid detection methods is desirable as a means of protecting the integrity of the food supply chain and populations from the effects of these Class 1 carcinogens. Aptamers are biomolecular ligands composed of nucleic acids. They can be selected to bind specifically to bacterial cells, viruses, proteins and smaller molecular targets such as pesticides, organic dyes and biotoxins. Once developed, they can subsequently be exploited similar to more traditional biomolecules such as antibodies. The initial experimental work focuses on optimising a novel method based on the selective exponential enrichment of ligands (SELEX). The procedure (capture SELEX) uses magnetic beads to capture the aptamers within an initial random pool that specifically bind to the target molecule. The advantage of this procedure is that the target molecule does not have to be bound to a solid support. As a result, problems such as recognition by the aptamers of either the bond used to link the small molecule target to the solid support or any linker molecules are avoided. Purification and separation steps to remove unwanted and non-binding aptamers are also simplified. The results presented confirm that the selection procedure is capable of producing aptamers with specificity for Type A trichothecenes.

Keywords: *mycotoxins, aptamers, SELEX*

C15
SELECTION OF OLIGONUCLEOTIDE APTAMERS FOR LISTERIA MONOCYTOGENES: NOVEL APPROACHES IN SELEX AND DETECTION METHODS FOR IMPROVED PERFORMANCE

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Aptamers are short oligonucleotides comprised of fixed primer binding regions and random sequence regions. Through a process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX), aptamers may be selected for a variety of target molecules such as proteins, peptides, toxins, viruses and whole cells. This effectively facilitates their use as biomolecular ligands similar to antibodies. Aptamers can offer several advantages over antibodies such as in vitro production thus avoiding ethical implications as with antibodies, ease of labelling with little or no alteration to binding sites, reduced production cost and longer shelf life through greater thermotolerance. Data are presented that illustrate performance optimisation of conventional SELEX procedures for *L. monocytogenes*. Novel methods that include either direct modification of the SELEX process or the smart use of ligands in molecular diagnostic methods are described. These methods have demonstrated an ability to improve the performance (target affinity and specificity) of oligonucleotide aptamers and contribute to improved sensitivity of derived assays. The effect of culture media on the recognition of L. monocytogenes by the aptamers is also reported.

Keywords: *aptamers, SELEX, L. monocytogenes*

C16
IDENTIFICATION OF DIFFERENT TYPES OF HONEYS BASED ON DNA AND PROTEIN COMPOSITION

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Our research aim was to verify the origin of the various domestic honeys in order to prevent sophistication. Therefore, those kinds of methods were developed, which were suitable to measure the pollen-protein and DNA present in different amount in the different types of honeys and the various mixture of different honey types. Protein isolation from Acacia honey was achieved by a so-called three-phase partitioning method (TPP). The TPP fractionation method is special in several respects, because it combines the effects of neutral salting-out and the organic solvent fractionation. The isolated proteins were separated by lab-on-a-chip (LOC) – and two-dimensional (2-DE) electrophoresis according to their molecular weight, and both molecular weight and isoelectric point, respectively. For the DNA-based tests, various appropriate isolation methods were developed in order to damage pollen cell wall and gain larger quantity and better quality of DNA extract. Subsequently, PCR method was developed to analyze acacia honey -being well-known as one of the most famous Hungarian honey- and linden honey as well. We observed that the TPP method has been adequately adaptable as a good separation technique for both isolate and concentrate proteins. The advantage of our self-developed method is the higher protein yield (100 %) comparing with other methods (50-80 %). During the development of DNA -based method, the most suitable isolation from the thick honey pollen cell wall was the enzymatic digestion, which was followed by Wizard isolation. The amplification of the isolated honey-DNA was tested by plant-specific primers (trnL), and then primers to amplify 107 bp-length fragment of nitrate reductase gene and 106 bp-length fragment of linden honey DNA were adapted. After the optimization of the PCR-method (number of cycles, primer and template concentrations, the binding temperature, etc.) in both cases, PCR analyses were successful for honeys.

Keywords: *three-phase partitioning fractionation, lab-on-a-chip, two-dimensional electrophoresis, PCR*

Acknowledgement: *This work was supported by the grant of the Research-Development-Innovation Project of the Hungarian Ministry of Rural Development*

C17
AN IMPEDIMETRIC APTASENSOR FOR THE DETERMINATION OF AFLATOXIN M1 IN MILK

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Aflatoxins constitute the most widely studied group of mycotoxins, they are mainly produced by fungi growing on vegetal products, mainly belonging to Aspergillus sp. Among aflatoxins, aflatoxin B1 (AFB1) has been designated as a primary carcinogenic compound by the International Agency for Research on Cancer (IARC). The presence of AFB1 in feed and the subsequent exposure of lactating animals lead to the contamination of milk by its hydroxylated metabolite, aflatoxin M1 (AFM1). Considering that AFM1 is included in the first group of environmental factors that can increase the risk of human cancer, most countries have set up maximum residue levels (MRL) of AFM1 in milk. In USA, China and Brazil, the maximum permitted level of AFM1 in milk has been set to 0.5 µg/kg. In European Community, much more stringent limits have been fixed through the European Commission Regulation 1881/2006, which sets a maximum limit of 0.05 µg/kg for AFM1 in milk products. Enzyme-linked immunosorbent assays (ELISA) have become very popular for mycotoxins analysis due to their many advantages such as sensitivity, high sample throughput, and need of small sample volumes. However, they are limited by their relatively elevated cost and by ethical issues, as immunoglobulins are produced through animal immunization. Among potential alternative recognition elements, aptamers have raised as promising tools for diagnostics, owing to their many advantages such as low cost, flexibility, ease of modification, high stability, and compatibility with large-scale production. In this work, an aptasensor was designed for the determination of aflatoxin M1 (AFM1) in milk based on DNA-aptamer recognition and electrochemical impedance spectroscopy (EIS) detection. A hexaethyleneglycol-modified 21-mer oligonucleotide was immobilized on a carbon screen-printed electrode through carbodiimide immobilization, after diazonium activation of the sensing surface. Cyclic volt ammetry and EIS were used to characterize each step of the aptasensor development. Aptamer-AFM1 interaction allowed the determination of AFM1 in buffer in the range 2-150 ng/L (LOD=1.15 ng/L). Application to milk analysis involved a preliminary filtration through a 0.2 µm PTFE membrane, allowing determination of AFM1 in milk for concentrations up to 1000 ng/kg. A linear response was observed for low concentrations ranging from 20 to 80 ng/kg. These performances are compatible with the levels set in European Union for milk and dairy products for adults (50 ng/kg) and infants (25 ng/kg).

Keywords: *aflatoxin M1, milk, aptamer, electrochemical impedance spectroscopy, biosensor*

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C18
DEVELOPMENT OF MANGALICA-SPECIFIC, RAPID DNA-METHOD

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Species-specific identification of livestock and game animal meat has an importance for medical, ecological, financial and religious reasons including the government regulation because consumer protection and food safety have received greater attention in recent years. In most cases, cheaper or less valuable meat of other species is added to the products. Many different analytical techniques have been developed and tested for species identification of meat, fish or dairy products, such as simple and real-time PCR methods. The application of these methods requires specialised environment with different accessories and equipments. Samples must be collected and transferred to the laboratory where qualitative and quantitative determination is performed. Our goal was to produce quick, easily portable in hand to detect Mangalica content is raw and processed food samples. The Mangalica is a Hungarian breed of domestic pig. Nowadays Mangalica population is considerably growing; due to the high quality of meat products are favoured in a particular cuisine, since tenderness and juiciness are positively correlated with intramuscular fat content and the type of fat. To achieve our purpose we have chosen recombinase polymerase amplification (RPA) to perform species specific reaction. It requires only a simple thermostat. RPA is an isothermal (constant temperature is around 38°C) amplification utilising strand displacing DNA polymerase and single strand binding recombinase. Among RPA applications with different probes, we tested Twist Amp nfo kit, which can be used with endpoint detection cost effectively. Endpoint detection was performed using lateral flow strip with easy yes/no indicator. As for sample preparation we were also seeking for minimal hands-on requirements. The applicability of the newly developed method was tested on several food samples.

Keywords: *mangalica, DNA, izothermal PCR*

C19
DIOXIN-LIKE COMPOUNDS IN LAKE FISH SPECIES: EVALUATION BY DR-CALUX BIOASSAY

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Diet in humans contributes more than 90% to the daily intake of dioxins and PCBs and fish products are considered the main contamination source. Exposure to dioxins and PCBs has also an impact on the immune system and on detoxification enzyme systems of humans and animals: in particular CYP1A oxidative enzyme in fishes can act as bioindicator in environmental monitoring. Currently, in northern Italy there are no projects monitoring lakes and fish farms in areas contaminated by industrial sources. The aim of this work was to evaluate dioxins and PCBs contamination levels in Piedmont and in neighboring areas considering two wild fish species (perch and roach) and a farmed species (rainbow trout) by using a screening cell bioassay for the detection of dioxin-like compounds in food, called DR-CALUX. It also had the aim to study a potential correlation between the level of dioxin like contamination and the health status of fish. Two lakes, Maggiore and Como, were chosen to collect perch and roach samples; furthermore, two different fish farms near the Lake Maggiore were selected to collect trout samples. A total of 134 samples were analyzed by DR-CALUX method. Muscle meat of fish was subjected to extraction of fats and purification into silica columns. For dioxin detection, rat hepatoma cells (H4IIE) genetically modified with the construct of the luciferase reporter gene were incubated with the samples extracts and the luciferase activity proportional to the dioxin amount was measured by chemiluminometer. All samples were subjected to anatomopathological, parasitological and bacteriological analysis. Moreover, in the case of rainbow trout, blood samples were submitted to hematological analysis and samples of liver were submitted to immunohistochemical detection of CYP1A. In order to detect differences between groups by sampling site and by species, a non-parametric test (Kruskal-Wallis) and two different linear regression models were applied. Statistical analysis was performed by the software Stata 13. All samples examined by DR-CALUX assay were compliant to EU Regulation, with values significantly lower than maximum level and action limit of respectively 6.5 and 3.5 pg WHO–PCDD/F-PCB TEQ/g/wet weight. Histological evaluations confirmed the absence of pathological changes due to toxic substances, referred to the amount detected. CYP1A antigen expression in rainbow trout liver samples was absent. By means of statistical analysis, a variability of results was found and it was related to species and site: Lake Maggiore perch fish had higher levels of dioxin-like contamination than roach and rainbow trout. On the basis of the results and regarding the species and contaminants under study, it is possible to affirm that there is no risk to the population in the consumption of the fish species deriving from this area.

Keywords: *dioxin-like compound, fish, biomonitoring, bioassay*

Acknowledgement: *This work was co-founded by Fondazione Cassa di Risparmio di Torino - CRT (grant nr. 2013 -1286)*

C20
SALMONELLA DETECTION FROM STOOL SAMPLES AND FOOD PRODUCTS BY USING A NOVEL, FAST AND SPECIFIC ISOTHERMAL AMPLIFICATION TECHNOLOGY, SIBA®

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Salmonellosis, caused by Salmonella enterica subsp. enterica, is one of the most commonly and widely distributed foodborne diseases. Salmonella carrying animals and humans cause considerable problems in primary production and food processing industry. We have developed a fast and accurate nucleic acid based detection system for Salmonella carriers with very low amounts of bacteria in their stool as an alternative to the widely used and time-consuming culture based method.

Methods We used a selective selenite broth for enrichment of Salmonella, and an isothermal DNA amplification method, Strand Invasion Based Amplification (SIBA®), for the detection of the bacteria in stool. The assay also contains an internal control in the multiplexed reaction. The workflow includes enrichment incubation and sample preparation, where 200 µl of enriched growth medium is transferred into a lysis reagent. After a filtration step, the sample is heated, mixed with reaction buffer and added to a tube containing lyophilised reagents. The assay is run on the Orion GenRead® instrument and detected with fluorescent readout together with an integrated qualitative algorithm and an internal amplification control. The sample preparation takes only around 10 minutes, followed by fast amplification of 50 min or less.

Results The doubling time of Salmonella in selenite medium was determined with and without stool. Our test was able to detect down to 1 CFU of Salmonella (as a starting material with stool sample) after only 8 hours of incubation. We also found Buffered peptone water (BPW) and Rappaport-Vassiliadis-Soypeptone (RVS), broths widely used in enrichment of environmental and food product samples, compatible with our test system.

Conclusions According to our preliminary results, Salmonella Carriage assay could offer fast Salmonella carriage screening after 8–10 hours incubation, easy sample preparation (30 min/12 samples) and 50 minutes Orion GenRead® run. With minor modifications, this system will also be suitable for testing environmental samples, animal stool, as well as food products, among others.

Keywords: *salmonella, DNA, human testing, pathogen, food testing*

C21
RAPID ANALYSIS OF L-MALIC ACID IN COMMERCIAL WINES AND REFERENCE SYNTHETIC WINES BY MEANS OF SCREEN PRINTED BIOSENSORS

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Determination of L-malic acid in wine is of great importance for the proper control of malolactic fermentation. There are many analytical methods that can be used to measure L-malic acid [1] including paper chromatography, HPLC, or capillary electrophoresis. However, all these methods are time-consuming and require highly trained technicians. FT–IR techniques [2] and enzymatic methods [3] are more often used; the first ones are less precise while the second ones are the most commonly used at the moment. Additionally, amperometric biosensors have recently emerged as a possible alternative for L-malic acid determination [4]. They are based on the measurement of the current intensity generated through a enzymatic redox reaction that, in non-saturating concentration of analyte, can be related to the analyte concentration. These biosensors are as specific as the enzymatic methods but have the advantage of being much faster since they do not require the reaction to be completed. Recently the research centre IK4-CIDETEC and the company BIOLAN Microbiosensors S.L. (Zamudio, Spain) have developed screen-printed biosensors that allow the determination of L-malic acid in about one minute. Additionally Biolan has developed a small device (BIOWINE 700) where the biosensors can be incorporated and provide directly the L-malic acid value for determination in wine samples. This work presents the results obtained for the determination of L-malic acid with this technology in reference synthetic wine solutions and in several commercial wines, compering the results with those obtained by the enzymatic method. The results indicate that the biosensor is comparable to the enzymatic analytical method, while the proposed biosensor measures L-malic acid in just a minute.

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Keywords: *L-malic acid determination, biosensor, commercial wines, screen printed biosensors*

C22
METABOLIC PROFILING AND QUALITY CONTROL ASPECTS OF OLIVE OIL USING AN FT-ICR-MS DIRECT INFUSION METHOD

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Olive oil is largely known for its nutritional and health beneficial effects. Furthermore, its economic value for European Union's economy is significant since EU comprises the first producer and exporter worldwide. However, what is not well-known is that olive oil tops the food ingredient fraud ranking according to FDA. Roughly more than 24% of olive oils distributed globally are fraudulent. Thus quality assurance and quality control methods are of high importance. So far numerous analytical methods have been employed towards this direction such as HPLC–UV, GC–MS, LC–MS and NMR. As a continuation of our work in olive oil, Fourier transform ion cyclotron resonance mass spectrometry (FT–ICR–MS) using direct infusion was employed. Due to the ultra-high mass resolution of FT–ICR–MS these complex sample can be analyzed without any chromatographic separation. The aim of the study is to map Greek olive oils as well as investigate the power of this methodology fo r quality control aspects. In brief, for the harvesting year 2014-2015 more than 150 samples of olive oil were collected from 10 geographical regions, 10 tree varieties, 9 harvesting periods, 4 cultivation methods and 3 different production procedures from all over Greece. Following detailed registration, all samples have been subjected to one-step liquid-liquid extraction for defatting purposes and were directly infused to the MS using "loop-injections" in ESI negative mode. The MS data were subjected after data pre-treatment to supervise and unsupervised multivariate data analyses methods. The initial results showed that the oils analyzed could be clustered according to the area of origin using especially OPLS–DA methods. Moreover, interesting trends were observed when cultivation practice was used as descriminant factor. According to our initial results, a direct infusion method using an FT–ICR–MS platform in combination with a metabolic profiling concept is a valuab le approach for olive oil quality control purposes.

Keywords: *olive oil, quality control, FT–ICR MS, Multivariate data analysis, mapping*

Acknowledgement: *OLA.LA PAVET PROJECT - 1471-BET-2013*

C23
INFLUENCE OF FERMENTATION TIME AND TYPES ON MICROBIAL COMMUNITIES ASSOCIATED TO COCOA BEANS USING PCR-DGGE

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Microbial fermentation is a crucial step in the post-harvest processing of cocoa and plays an important role in global and organoleptic quality of chocolate. The fermentation process is applied differently according to the country and farmer. The present study aims to investigate the influence of methods and fermentation time on the microbial communities structure associated with cocoa. The cocoa beans fermented during six days by three different methods in Ivory Coast (fermentation in wooden boxes, in plastic boxes and heap) were studied by global analysis of microbial ecology using Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis (PCR–DGGE). This molecular technique was associated with sequencing step in order to identify microbial genera. The samples were collected at three time's intervals: 2, 4 and 6 days of fermentation. The results of this study showed that DGGE profiles obtained from the fermented beans by three methods were similar while they presented more variations in the microbial communities during fermentation time. The structure of microbial communities varied according to the fermentation time and not much following the fermentation method. PCR-DGGE was an effective tool to discriminate cocoa post-harvest practices.

Keywords: *microbial communities, cocoa, fermentation process, PCR-DGGE*

C24
A NEW ENZYME-FREE ELECTROCHEMICAL IMMUNOASSAY FOR EXPRESS DETECTION OF INFECTIOUS SPECIES

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The modern world has witnessed a steady increase in the incidence of infectious diseases caused by bacterial contamination of water and food. Some strains of bacteria can cause such serious diseases as dysentery, meningitis, peritonitis and sepsis. In this connection, there is an urgent need for rapid screening techniques for water and food, as well as for differential diagnostics of infectious diseases at an early stage. Undoubtedly, existing approaches to detecting pathogens in test samples – in particular, enzyme immunoassay (ELISA), polymerase chain reaction and bacteriologic culture techniques – all have their advantages. However, these methods are often time-consuming and complicated as well as requiring advanced and costly instrumentation. This paper presents a new enzyme-free electrochemical immunoassay for *Escherichia coli* (ATCC 25922) detection. The immunoassay involves the formation of an immunocomplex between the antibodies immobilised on the surface of electrode, and bacteria, labelled magnetite nanoparticles. We used two types of nanoparticles as a signal label in the immunoassay: 1. magnetic nanoparticles Fe3O4; 2. magnetite nanoparticles surface modified with a variety of electroactive organic compounds: polypyrrole, polyvinylbenzylchloride with quinoline, silica oxide with ferrocene. The main novelty is the realisation of a procedure for the detection of an electrochemical signal from a magnetic nanolabel included within the immunocomplex on the electrode surface. The developed immunosensor is highly specific, enabling the detection of E. coli at a concentration range from 10 to 105 colony-forming unit (CFU)/mL with a relative standard deviation (RSD) of less than 10%. The detection limit for the proposed electrochemical immunosensor is 9.3 CFU/mL. Bacteria have been detected in model mixtures as well as in real samples. Recovery of the method was observed in the range of 95–99%, a figure that is comparable with the ELISA test.

Keywords: *immunoassay, nanoparticles, Escherichia coli*

C25
OPTIMISED ASSAY OF ENDOTHELIN-CONVERTING ENZYME-1 INHIBITION IN HAM BY-PRODUCTS

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Proteolysis constitutes one of the most important biochemical reactions that occur during dry-cured ham processing, and is responsible for the main changes in texture but also for the generation of thousands of small peptides which influence the characteristic flavor of the final product. Some of the sequences of these naturally generated peptides have also been described as bioactive because they exert antioxidant or antihypertensive activity. In this sense, biologically active peptides are usually short, between 3 and 15 amino acid residues, and are released from food proteins during digestion or processing. Meat industry produces large amounts of by-products that represent both an economical and environmental problem, being dry-cured ham manufacturers important producers of by-products including all residues derived from the deboning and slicing of ham such as rinds, bones, skin, etc. These by-products may have an added value since they can constitute an interesting source of bioactive peptides. Recent research studies to reduce hypertension which is one of the major risk factors for the development of cardiovascular diseases, stroke, and end-stage renal disease, are mostly based on naturally generated antihypertensive peptides which results less aggressive, reducing the secondary effects of antihypertensive drugs like captopril. Angiotensin-converting enzyme inhibitory peptides have been the most studied for the moment in food matrices but blood pressure is very complex and there are many other metabolic pathways involved in the control of vasoconstriction. This is the case of endothelin-converting enzyme-1 (ECE-1) that is a membrane-bound zinc-metallopeptidase with amino acid sequence related to neprilysin. A major in vivo function of ECE-1 is the proteolytic conversion of big endothelin-1 to endothelin-1, one of the most potent vasconstricting peptides known. The inhibition of ECE-1 should result in the reduction of systolic blood pressure but only few studies hav e focused on the potential effect of food-derived peptides on the ECE-1 system, and no studies of ECE-1 inhibitory activity have been described in meat products. In this study, the methodology for the assay of ECE-1 inhibition has been optimised and different positive controls have been evaluated with phosphoramidon showing the lowest IC50 with a value of 1.16 nM. The optimised method has been tested with peptide extracts obtained from dry-cured ham by-products and fractionated through size-exclusion chromatography. The obtained fractions were assayed for ECE-1 inhibitory activity and some of them showed inhibition values near 100%.

Keywords: *bioactive peptides, antihypertensive peptides, endothelin-converting enzyme-1, enzyme assay, ham by-products*

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C26
TRACING OF NUTRITIONAL ASPECTS IN HUMAN BLOOD SERUM BY UHPLC–QORBITRAP MS

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Statins belong to the class of hypolipidemic drugs mainly used to treat high levels of cholesterol in blood. They act as competitive inhibitors of a HMG-CoA reductase, enzyme limiting the rate of cholesterol synthesis pathway. This inhibition not only leads to depletion of cholesterol and its esters with fatty acids, but also to depletion of intermediates of this metabolic pathway (farnesyl- and geranylgeranyl pyrophosphate), which can play important role in tumor proliferation. Knowledge of concentrations of all these compounds may thus serve for better assessment of the overall effect of statins on treated patients. The main analytical challenge of the study was to increase the method throughput by integrating of all the mentioned analytes into the one determinative step. This was definitely not an easy task because of different physico-chemical properties of all compounds of interest (8 semipolar statins, and their biologically active metabolites, 2 polar intermediates of cholesterol synthesis, and relatively non-polar cholesterol and its 3 main esters with fatty acids). Various ways of sample preparation were examined in order to minimize the content of the blood serum proteins, and assure good recoveries and low matrix effect for these analytes. The separation by ultra-high performance liquid chromatography coupled with tandem high resolution mass spectrometry (UHPLC–HRMS/MS) method with Q-orbitrap as mass analyzer was performed. The potential of Q-orbitrap mass spectrometry, i.e. the sensitivity and the broad dynamic range, were fully exploited for analysis of these heterogenous com pounds differing also in their natural concentration in blood, thus in demands on the analytical performance. The troubleshooting we met during the method development, as well as the final performance characteristics, will be discussed in details.

Keywords: *blood serum, nutrition, high resolution mass spectrometry*

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C27
MINIMUM PERFORMANCE PARAMETERS FOR MOLECULAR ANALYTICAL METHODS

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The use of molecular analytical methods across diverse sectors has exploded over the last decades. Yet, there is a lack of clear and harmonized criteria to define whether methods are reliable (of high quality) or not. Numerous methods published in scientific journals were developed with only one pre-defined performance goal: some degree of specificity. Many methods used routinely for various purposes have not been properly validated, due to lack of pre-defined performance thresholds. The result is potential waste of resources, development of inferior methods or even worse, routine application of methods that are not reliable. Furthermore, there is surprisingly little cross sectorial dialogue concerning performance criteria. Molecular analytical techniques, with exception for sample preparation and nucleic acid extraction and purification, are almost identical from a technical point of view. One of the main activities of the Decathlon project (www.decathlon-project.eu/) is to establish minimum performance parameters (MPPs) for molecular analytical methods with cross sectorial applicability, covering nucleic acid extraction, nucleic acid amplification and next generation sequencing. An MPP is an identified, necessary checkpoint for an analytical method. An associated acceptance value (AAV) is the critical (threshold) value for a specific MPP that the performance must comply with. Properly defined MPPs and AAVs are expected to increase the general quality of molecular analytical tools and faster replacement of underperforming methods with better methods. A method can then be benchmarked against these MPPs and AAVs. Failure to meet the AAV for a specific MPP means that the method is underperforming on this particular quality criterion. It is possible to develop a method without consideration of MPP and AAV, and then assess its performance. This, however, can reduce the chance of developing a method that is fit for successive use. M PPs are applicable at all stages in the life history of the analytical method: development, validation and routine application. The prioritization of MPPs can be purpose dependent. AAVs sometimes have to be sector or even application specific, e.g. due to specific legislation or purpose. A first set of MPPs and AAVs were prepared by the project partners late in 2014. These can be downloaded from the project website or obtained from the project coordinator. The preparation of a final, amended and expanded set of MPPs with AAVs to be launched late in 2016 is ongoing. Achieving MPPs truly applicable across sectors depends on efficient cross sectorial stakeholder dialogue. We therefore invite interested stakeholders to enter the dialogue, in particular those who represent sectors not already well represented in Decathlon. There is a lot to contribute, we have a lot to learn, and there is a lot in it for all users of molecular analytical methods.

Keywords: *DNA extraction, amplification methods, next generation sequencing, method performance, benchmarking*
Acknowledgement: *Financial contribution from the European Commission (contract no. FP7-KBBE-2013-7-613908), China National Transgenic Plant Special Fund (2014ZX08012-002), the Norwegian Research Council, the Dutch Ministry of Economic Affairs and the Slovenian Research Agency (contract P4-0165)*

BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

(D1 – D34)

D1
ANTIBACTERIAL ACTIVITY OF PAPAIN HYDROLYSED CAMEL MILK WHEY

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Camel milk exhibits a wide range of biological properties including, antioxidative, antithrombotic, antihypertensive and immune-modulatory effects. The aim of the present study was to isolate and identify peptides from camel milk whey (CMW) with antibacterial activity against *Staphylococus aureus* and *Escherichia coli*. CMW was hydrolysed with papain for 4 hours and the hydrolysate was fractionated by size exclusion chromatography (SEC) and assessed for antibacterial activity. The active fraction was further purified by RP-HPLC (C18 column), resulting in great peak separation. Peptides from the most active fraction were identified using a UHPLC+ Ultimate 3000, coupled with Finnigan LTQ mass spectrometer. Antibacterial activity of the SEC fractions was investigated against *S. aureus* and *E. coli* using a standard disc diffusion assay methods yielded one antibacterial fraction. The minimal inhibition concentration of this fraction against S. aureus and *E. coli* was 0.01 mg/ml and 0.39 mg/ml, respectively. Transmission electron microscopy (TEM) was used to visualize the morphological and ultrastructural changes of the treated pathogens compared with the untreated controls. In conclusion, we have demonstrated that hydrolysis of camel milk whey with papain generates small and extremely potent antimicrobial peptides against *S. aureus* and *E. coli*. These results are very promising for the future use of these structures as food preservatives or antibacterial lead drug candidates.

Keywords: *camel milk whey, antibacterial peptides, papain*

D2
REVERSED PHASE×REVERSED PHASE LIQUID CHROMATOGRAPHY AS A POWERFUL ANALYTICAL IN THE ANALYSIS OF COMPLEX FOOD SAMPLES

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One-dimensional chromatography is the most widely applied technique to the analysis of real-world samples in several fields. However, whenever the complexity of the sample overwhelms the separation capability attained by a single separation system, more powerful analytical techniques must be used for achieving rewarding results. Comprehensive two-dimensional liquid chromatography (LC×LC) is a technique of great analytical impact since it offers much higher peak capacities than separations in a single dimension. Combination of normal phase (NP) and reversed phase (RP) LC separation systems is one of the most orthogonal approaches in LC×LC, whereas the coupling of two RP phases provides considerably lower peak capacity values due to the partial correlation of the two dimensions. In this contribution, the use of different gradients strategies in RP-LC×RP-LC allowed to properly tune orthogonality, thus increasing the LC×LC separation space, despite the use of partially correlated stationary phases. Selectivity correlation plots were investigated using different combinations of columns (cyano, amide, C8 and C18 stationary phases) and mobile phases (methanol, ethanol or acetonitrile as phase B). Depending upon the sample to be analyzed combinations of cyano×C18 for sugarcane and red wine, and amide×C8 for biomasses were successfully employed. From a detection viewpoint, photodiode array (PDA) and mass spectrometry (MS) techniques were successfully employed for structure elucidation, representing an effective third added dimension to the LC×LC system.

Keywords: *comprehensive chromatography, food, selectivity*

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D3
STUDY OF CATECHIN DEGRADATION IN GREEN TEA BY FAST GRADIENT HPLC/MS

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Tea is very popular and widely used due to its beneficial effects on human health. Green tea contains high amount of phenolic compounds with antioxidant properties, especially catechins, from which the epigallocatechin-3-gallate (EGCG) is the most important and widely studied compound. This work is focused on optimization of fast chromatographic separation of catechins and other phenolic compounds presented in green tea using liquid chromatography coupled with mass spectrometry in multiple reaction monitoring mode. Chromatographic column packed with porous shell particles and the mixture of methanol and water with addition of formic acid as a mobile phase was used for separation. All green tea catechins have been separated under optimal conditions in two minutes, which allowed us to study a degradation of catechins during long stand period of green tea infusions prepared by water differing in temperature. Both, total polyphenolic content and antioxidant acti vity were determined using spectrophotometric methods in all infusions. Significant differences of catechin contents were found between infusions in dependence on temperature of water and during long stand period of green tea infusion. Decreasing antioxidant activity and total polyphenolic content were proven during storage. Significant decrease of EGCG and epigallocatechin concentration was observed during long stand period of green tea infusion. The degradation products are probably gallic acid, catechin or epicatechin, which content significantly increased.

Keywords: *green tea, catechins, liquid chromatography, mass spectrometry*

D4
STABILITY STUDY OF PHYTOCHEMICALS IN NUTRACEUTICAL PRODUCTS APPLYING HIGH RESOLUTION MASS SPECTROMETRY

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The consumption of nutraceutical products mostly derived from plants has significantly grown in the last few years. This is mainly due to the large number of beneficial properties attributed to the high content of bioactive compounds in these products. In this sense, capsules from green tea, royal jelly, soy or grapes are widely consumed. Several kinds of families of bioactive compounds are characteristic in each matrix, such as catechins in green tea, isoflavones in soy or resveratrol, anthocyanins and procyanidins in grapes. During the storage of these products, the stability of bioactive compounds must be considered because this can affect the benefits associated to these products. Nevertheless this fact has been scarcely studied in nutraceutical products [1], due to the common assumption that dry products might be inherently stable. In this study, a database containing more than 50 bioactive compounds has been used to evaluate the stability of these analytes in different types of nutraceutical products based on plants and other products (grape, tea, royal jelly and soy) during one year storage at 5°C. To determine these compounds, liquid chromatography coupled to high resolution mass spectrometry (LC–HRMS) (Exactive-Orbitrap analyzer) was applied. This analyzer offers the possibility of performing retrospective analysis (detection of compounds not included in a target list) and operate in full-scan mode, and theoretically unlimited compounds can be monitored. In relation to degradation products, they were identified after 3 months of storage in green tea (coumestrol, formononetin and pinocembrin) and soy products (formononetin and coumestrol). In the case of royal jelly, quercetin, pinocembrin and isorhamnetin/tamarixetin were detected after 6 months of storage. Finally no degradation products were identified in grape-based samples.

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Keywords: *nutraceuticals, phytochemicals, stability, high resolution mass spectrometry*

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D5
NUTRITIONAL EVALUATION AND BIOLOGICAL POTENTIAL OF SCANDIX PECTEN-VENERIS

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Scandix pecten-veneris, commonly known as Shepherd's needle. This plant was not explored for nutritional and biological potentials. The aim of present work was to evaluate the nutritional chemical composition and biological potential of *S. pecten-veneris*. The leaves of the selected plant leaves were assessed for nutritional, anti-nutritional, antioxidant and antimicrobial activities. Mineral profile was determined by atomic absorption spectrophotometer, antimicrobial activities were determined against eight common bacteria, antioxidant activity was tested using the persistent free radical 2,2-diphenyl-1-picryl hydrazyl (DPPH), while antiradical activity was examined by RPA assay. It was found that the plant contain comparable quantity of moisture, ash, lipid, fiber, protein, carbohydrate, vitamins and essential minerals with the computed energy of 417.42±15.53 kcal/100 g dry weight. Among the anti-nutritional components, oxalic acid and phytic acid found at low concentration. S. pecten-veneris showed a moderate antiradical activity, reducing quantity of produced hydroxyl radicals to about 20% of initial value. DPPH radical scavenging activity of different extracts of plant showed a linear correlation with total phenolics in the order of water > n-butanol > chloroform > ethyl acetate > methanol. Total antioxidant activity of methanolic extract was lower than vitamin E. Reducing power assay of different extracts was lower than vitamin C, while greater than vitamin E. Regarding the antimicrobial bioassay; S. pecten-veneris leaves showed highest zone of inhibition against *Staphylococcus aureus and Candida albicans*. The results showed that *S. pecten-veneris* is an important unconventional edible plant with enough nutrients, antioxidant and antibacterial properties.

Keywords: *Scandix pecten-veneris, nutritional evaluation, biological potential*

D6
OILSEED CAKE POTENTIAL AS A HEALTH PROMOTING FOOD COMPONENT

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Oilseed cakes are by-products of oil-extracting process and they are normally used as fodder in animal nutrition or as a fuel in heating plants. From an economical point oilseed cakes seem to be underutilized as they provide an excellent source of raw material with potential applications as nutraceuticals and health promoting food components which is in line with recent food market trends that search for products rich in omega-3 fatty acids, dietary fiber or substances with antioxidative properties. Therefore, the present research focuses on the antioxidant potential of selected oilseed residues received after oil cold-pressing. The aim of the study was to compare the antioxidant activity of extracts obtained from defatted cakes of three different oilseeds (flaxseed, evening primrose and black cumin). The influence of the extraction procedure on the total phenolic content (TPC) in the extracts and on their antioxidant activity was investigated. When exploring the antioxidant potential of different extracts, the use of a single test is insufficient to identify the different mechanisms involved. Therefore, to evaluate the antioxidant potential the two antioxidant assays, namely the reducing power assay (FRAP) and DPPH-radical scavenging activity, were performed. Moreover, the efficiency of obtained extracts in promoting the oxidative stability of cold-pressed flaxseed oil samples incubated in elevated temperature was assessed. Three different concentrations of each extract (500, 1000 and 2000 ppm) were applied to the oil samples and the content of primary and secondary oxidation products was measured. The extracts from defatted oilseed cakes showed relatively high total phenolic content and the antioxidant activity in model assays. The highest TPC and antioxidant potential exhibited extract obtained from evening primrose cake. The addition of extracts to the flaxseed oil delayed the oxidative changes in selected cases and was dependent on the concentration used. The highest protective efficiency exhibited an extract obtained from flaxseed cake (2000 ppm addition) which might be used as an alternative natural source of antioxidants for flaxseed oil stabilization.

Keywords: *oilseed cake, antioxidant potential, health promoting food components*

D7
COOKED EDIBLE GREENS OF GREECE: "CHÓRTA" AND THEIR DECOCTIONS AS SOURCES OF VALUABLE WATER-SOLUBLE PHYTONUTRIENTS

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The Greek diet as a part of the Mediterranean diet has provided alternative sources of phytonutrients with unique properties. Wild or semi-cultivated cooked edible greens (chórta), substitute for the more conventional leafy salads in a regular basis.1 Taxonomically, chórta do not share botanical uniformity and comprise a subset of edible plants mostly belonging to the family Asteraceae. Besides nutritional value, chórta have delivered solutions for therapy throughout the centuries, with vast ehtnopharmacological use.2 In specific, the decoctions resulting from chórta cooked salad preparations, are supposed to possess anti-diabetic, liver detoxifying and anti-inflammatory properties.2 Following the traditional Greek regime, we have investigated the chemical properties of the water decoctions of several chórta species, namely Cichorium intybus (Greek: radíki), Cichorium spinosum (Greek: stamnagáthi), Sonchus asper (Greek: sokhós), Centaurea raphanina (Greek: agkinaráki), Amaranthus blitum (Greek: vlíto), Scolymus hispanicus (Greek: askólimpros) and Crithmum maritimum (Greek: krítamo). UHPLC–HRMS qualitative fingerprinting with the aid of LTQ–Orbitrap XL platform has shed light into the chemical nature of the decoctions constituency. A combination of separation techniques such as adsorption and molecular exclusion resin chromatography and Fast Centrifugal Partition Chromatography was used to isolate constituents such as phenylpropanoids (cichoric and caftaric acid), chlorogenic acids, nucleosides, simple carboxylic acids, sesquiterpene lactones and flavonoids. Quantitation of important bioactive phenols such as cichoric and caftaric acid has been performed in the water decoctions, showing that the regular consumption of chórta cooking water as a traditional health regime followed in Greece can provide a plethora of phytonutrients. This abundance of chemodiversity can lead to the development of new functional foods and supplements with chemoprotective properties inspired by the Greek and Mediterranean diets.

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Keywords: *asteraceae, LC-MS profiling, FCPC isolation*

D8
RESPONSE SURFACE METHODOLOGY FOR HYDROLYSIS OPTIMIZATION TO OBTAIN EGGSHELL MEMBRANE PEPTIDE CONCENTRATES WITH BIOLOGICAL ACTIVITY

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Over the years, hen's eggs consumption has been increasing worldwide, including in Portugal. This increment comprises both industrial and household leading to an eggshell accumulation that can be responsible for environmental and public health issues. Eggshell treatment has become imperative to minimize these effects, as well as to find sustainable applications. Protein is the biggest constituent of eggshell membranes and, besides its high nutritional value, it plays a crucial role in some physiological functions due to the presence of bioactive peptides. So, in order to explore a new application for the eggshell membrane in food industry, a protein concentrate obtained from the former (100 % protein content at 40 g L^{-1}) was subjected to an enzymatic hydrolysis with alcalase from Bacillus licheniformis, viscozyme L and protease from Bacillus amyloliquefaciens. Hydrolysis conditions for proteins were optimized using response surface methodology. For each enzyme, experiments were conducted with two independent variables, enzyme/substrate (E/S; %, *v/v*) ratio and reaction time (hours) whereas response used in experimental designs was the degree of hydrolysis (DH), angiotensin converting enzyme (ACE)-inhibitory and antioxidant activities (Oxygen radical absorbance capacity – ORAC – method) of the corresponding hydrolysates. Aiming to achieve that propose the central composite design consisted in a complete 22 factorial design, with thirteen independent experiments. Optimum conditions were determined by surface response, being r eaction time and E/S ratio used as parameters and DH, ACE-inhibitory activity and antioxidant activity used as objective functions. The suggested model showed to be statistically suitable to describe DH, ACE-inhibitory activity and antioxidant activity of hydrolysates. The optimum conditions observed for each hydrolysis considering the studied biological activities were: 6 h, 2.2% (*v/v*) for alcalase; 6.6 h, 1.9% (*v/v*) for viscozyme L; and 5.3 h, 2.9% (*v/v*) for protease. The best results were presented by alcalase hydrolysates. For optimum conditions alcalase hydrolysates presented ACE-inhibitory (IC50) of 34.5±2.1 μgmL^{-1}, while in viscozyme L and protease hydrolysates verified an IC50 of 63.0±4.2 μg mL^{-1} and 43.0± 8.5 μg mL^{-1}, respectively. Concerning antioxidant activity, values of 4.2±0.2, 4.4±0.1 and 3.8 ± 0.2 μmolTrolox equivalent mg^{-1} hydrolyzed protein were observed for alcalase, viscozyme L and protease hydrolysis, respectively. This work showed that eggshell membrane is an added-value by-product, since it is a precursor of biological activities and may have potential industrial application as a functional ingredient.

Keywords: *eggshell membrane, bioactive peptides, hydrolysis, ACE-inhibitory activityantioxidant activity, antioxidant activity (ORAC method)*

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D9
ANTIOXIDANT PROTECTION OF BRAZILIAN RED WINES IN SACCHAROMYCES CEREVISIAE

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Moderate consumption of red wine reduces the risk of cancer, heart and age-associated diseases, because the wine contains antioxidants that protect cells from oxidative damage arising from metabolic and exogenous sources. This is due to the wine's phenolic compounds, which act to maintain balance between the levels of reactive oxygen species (ROS) and cellular antioxidant defenses. In this study, the capacity of Brazilian red wines to protect different strains of Saccharomyces cerevisiae against oxidative stress caused by hydrogen peroxide was evaluated. Twenty samples of red wine of the Merlot variety produced in the South of Brazil were filtered and the total phenolic content was determined. The Wild-type strain of *Saccharomyces cerevisiae* EG103 (MATα leu2Δ0 his3-Δ1 trp1-289 ura3-52) and its isogenic mutants ctt1Δ, sod2Δ, sod1Δsod2Δ, sod1Δctt1Δ, containing, respectively, the genes CTT1 (cytosolic catalase), SOD2 (mitochondrial superoxide dismuta se), SOD1SOD2 (cytosolic and mitochondrial superoxide dismutase), SOD1CTT1 (cytosolic superoxide dismutase and catalase) interrupted, were used. To check resistance of the strains to oxidative stress, yeast cells in exponential growing phase, were inoculated in PBS 1X, pretreated with red wine for 60 minutes and subsequently exposed to 2 mM H_2O_2 for 60 minutes. Cell viability was determined qualitatively on YPD medium plates, inoculated in spots and subsequent incubation at 28°C for 72 hours. Cells of the wild-type strain acquired tolerance to oxidation when preadapted with red wine as well as cells lacking sod2. For strains lacking sod1sod2 and sod1ctt1 antioxidant protection was observed when samples of red wine had a higher phenolic content than 370 mg L^{-1}. The H_2O_2 generates highly reactive and toxic hydroxyl radicals, which generally induce oxidative damage to biomolecules such as lipids, proteins and DNA. Wine phenolic compounds can module the cell response by inter acting with a wide spectrum of molecular targets, such as antioxidant enzymes. The wines with the highest concentrations of phenolic compounds reduced the potential for oxidative injuries in the cells of all the mutant strains. With the exception of yeasts lacking ctt1, wherein the specific antioxidant system was only able to acquire tolerance after treatment with wines with low polyphenol contents. Catalase activity is very low in fermentative cells but increases linearly with the increment in H2O2 concentration, which keeps the intracellular control of the oxidizing agent. Therefore, the treatment of the yeast cells with wine with phenolic content between 280 and 300 mg L^{-1} induced adaptive response, protecting cells from lethal effects of higher oxidant concentrations. These results suggest that high levels of ROS might be reduced following treatment of the cells with red wine.

Keywords: *Brazilian red wines, antioxidant protection, phenolic compounds, antioxidant enzymes, Saccharomyces cerevisiae*

Acknowledgement: *CAPES*

D10
ANALYSIS OF VARIOUS CAROTENOIDS FROM DIFFERENT COLORED PAPRIKA USING UPLC AND OPTIMIZATION OF CAPSANTHIN EXTRACTION FROM RED PAPRIKA USING ASE BY RSM

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In this study, we analyzed 11 kinds of carotenoids (neoxanthin, capsorubin, violaxanthin, capsanthin, zeaxanthin, lutein, α-cryptoxanthin, β-cryptoxanthin, antheraxanthin, α-carotene, β-carotene) from different colored paprika using UPLC equipped with a HSS T3 column. We improved the peak resolution and performed carotenoid analysis within 30 min. We validated the precision and accuracy of capsanthin in UPLC method. Capsanthin showed good linearity (R^2=0.9998) in the range of 1-200 μg/mL with 2.4 μg/mL and 7.2 μg/mL of LOD (limit of detection) and LOQ (limit of quantification), respectively. The relative standard deviation (RSD) for intra- and inter-day precision was less than 3.83%. Recovery was in the range of 91.86~99.87%. For the recovery of carotenoids from paprika using advanced techniques like accelerated solvent extraction (ASE), we performed capsanthin (CST) extraction from red paprika using ASE by Box-Behnken Design (BBD) in response surface metho dology (RSM). Three independent variables including temperature (°C, X1), static time (min, X2) and ratios of acetone and ethanol (*v/v*) (%, X3) were studied and CST contents as response variable was determined by UPLC. The data fitting reveled coefficient of determination (R^2) for the total model was 80.20% (p0.05). A mathematical relationship, $Y = 32.58 + 7.30X_1 + 2.00X_2 + 2.50X_3 - 2.36X_{12} + 4.10X_{22} + 3.71X_{32} - 2.89X1X2 - 4.02X_1X_3 - 5.09X2X3$, was obtained to explain the effects of all factors. The optimum extraction conditions were found to be 100°C, 5 min and 50% acetone/EtOH. Under these conditions, the mean extraction yield of CST was 26.12 μg/100 g dry weight, which was in good agreement with the predicted model value. The experimental values under optimal conditions were in good consistent with predicted values.

Keywords: *UPLC, carotenoids, response suface methodology, accelerated solvent extraction*

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D11
OPTIMIZATION OF LUTEIN RECOVERY FROM PAPRIKA LEAVES USING ACCELERATED SOLVENT EXTRACTION BY RESPONSE SURFACE METHODOLOGY

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In this study, extraction conditions for recovering lutein from paprika leaves was optimized using ASE designed by RSM and lutein contents were quantitatively analyzed using UPLC equipped with BEH C18 column. A central composite design (CCD) was employed for experimental design to obtain the optimized combination of extraction temperature (°C), static time (min), and solvent (EtOH, %), and the experimental data obtained from twenty samples set were fitted to a second-order polynomial equation using multiple regression analysis, quantitatively. The adjusted coefficient of determination (R^2) for the lutein extraction model was 0.9518, and the probability value (p=0.0000) demonstrated a high significance for the regression model. The optimum extraction condition of lutein was found to be at the following values: temperature 93.26°C, static time 5 min, and solvent EtOH 79.63%. Under these conditions, the predicted extraction yield of lutein was 232.6021 μg/g.

Keywords: *paprika leaves, accelerated solvent extraction, response surface method, UPLC, lutein*

D12
ANALYSIS OF VITAMIN B COMPLEX IN INFANT FORMULA SAMPLES BY LC–MS/MS

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The vitamin B complex is a group of water-soluble vitamins that play important roles in cell metabolism. The absence of individual dietary B vitamins can lead to several conditions, including depression and high blood pressure so they are often added to foods, especially infant formula. Human daily nutritional recommendations for the members of the vitamin B complex vary considerably, for example from 6 μg of vitamin B12 to 20 mg of vitamin B3. A rapid, robust, sensitive and specific LC–MS/MS assay using the SCIEX QTRAP® 6500 system has been developed for the simultaneous detection of all major forms of vitamin B complex. The method detects all currently used forms of vitamin B6 and vitamin B3 in infant formula and includes vitamin B12. The sample preparation allows the same extract to be used for Vitamin C detection and the LC–MS/MS conditions have been tuned so that the response for each vitamin is linear over the required detection ranges. Using a simple sample preparation has proved a valid approach to detect all of the fortified B vitamins in NIST 1849a infant formula. The NIST 1849A infant formula reference material results demonstrate the validity of this method. Results with excellent accuracy and reproducibility were achieved. The method was validated across three laboratories. Reproducibility of results was well below 20%.

Keywords: *vitamin B, LC-MS/MS, infant formula, NIST, multi-lab validation*

D13
CUCURBITACINS — A SOURCE OF DANGER

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In August 2015, there was a newspaper report of three cases of acute poisoning, one of them fatal, after the consumption of bitter-tasting zucchini. Responsible for this were the naturally occurring cucurbitacins. Cucurbitacins are highly oxidized tetracyclic triterpenes which are nearly exclusively present in plants of the family of the Cucurbitaceae, namely squash plants. Dependent on the dosage, they cause severe bloddy diarrhea, strong colic, nephritis, tachycardia, and circulatory, even leading to death. Nevertheless, they have also been assigned, e.g., anti-diabetic, hepato-protective, anti-inflammatory, and anti-tumoral effects. Due to these properties, cucurbitacins have come into the focus of research. Only a few of the approx. 80 cucurbitacins known are commercially available. They differ only little in their structure, sometimes only in stereochemistry, so that MS-detection is not really helpful. Therefore, we used the medically applied cucurbitacin-rich colocynth, the medical plant of the year 2012, as a source for isolating individual cucurbitacins as standard substances [1,2]. Numerous fruits and, to some extent, the leaves, seeds, and blossoms of plants belonging to the Cucurbitaceae family as well as of some other families described as possibly containing cucurbitacins were analyzed with regard to their cucurbitacin composition using HPLC–DAD and LC–MS/MS. As expected, no cucurbitacins could be detected in the flesh of commercial fruits and, for example, in the hot-bitter nasturtium blossoms used as edible decoration on salads. Only small amounts could be determined for bitter-tasting cucumbers. However, alarmingly high amounts of cucurbitacins were analyzed in three bitter squashes from private cultivation derived from plants grown from seeds that were harvested in the previous year.

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Keywords: *cucurbitacin, cucurbitaceae, squash, cucumber, nasturtium*

D14
SELECTIVITY OF SEPARATION OF NATURAL ANTIOXIDANTS IN GRADIENT REVERSED-PHASE LIQUID CHROMATOGRAPHY

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Polyphenols are a large family of compounds, including phenolic acids, flavonoids and tannins, widely distributed in plants and related food products. Many of them are natural antioxidants, which have beneficial effects on human health, by decreasing the level of free radicals in the organism; other polyphenols are toxic, harmful or even may have carcinogenic and mutagenic effects. These compounds also influence taste, flavor, color and durability of beer, wine, tea, fruit juice and other beverages. The polyphenols can be easily analyzed using liquid chromatography in reversed-phase mode; however, the optimization of separation selectivity is usually required especially for structurally similar or isomeric compounds. The purpose of the present work was to develop a systematic approach to the selection of columns and mobile phases suitable for separation of natural phenolic antioxidants, based on the correlations between the gradient retention times and molec ular structure-descriptors of representative phenolic acid and flavonoid standards. For this aim, multiple linear regression, cluster analysis and window–diagram optimization strategies are combined. The gradient retention data for a set of phenolic compounds obtained using six different columns were used to establish the retention model. Predicted retention factors were then applied for evaluation of selectivity using linear free energy relationship concept, which enables comparison of selectivity of separation among different stationary phases used. Finally, the best predicted conditions were verified by analysis of natural antioxidants in real matrices.

Keywords: *phenolic compounds, reversed-phase liquid chromatography, gradient elution, selectivity, retention model*

D15
DEVELOPMENT AND VALIDATION OF A NEW METHOD FOR EXTRACTION OF CAROTENOIDS FROM LYOPHILIZED ORANGE PULP

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Citriculture is an important economic activity for several countries, including Brazil, the main worldwide orange producer and exporter. Among citrus, oranges (*Citrus sinensis*) are the most produced fruit. Orange presents one of the most complex carotenoid profile, with the largest number of compounds among several fruits. Additionally, orange pulp is rich in epoxycarotenoids, which are unstable under the inherent matrix acidity. Therefore, carotenoid analysis in orange is still a challenge. Here we developed and validated a new methodology for carotenoid extraction from lyophilized orange pulp. We compared extraction through maceration using mortar and pestle with magnetic stirring, as well the effectiveness of using a mild neutralizing agent previously to extraction. Several combinations of ethyl acetate and methanol were tested. The results of total carotenoid contents, quantified by UV-visible spectrophotometry, were compared. The best results were obtai ned by the magnetic stirring procedure, using two extraction steps with ethyl acetate followed by two extractions with methanol, as well as Na_2CO_3 addition previously to extraction to neutralize organic acids. After extraction, the carotenoids were transferred to diethyl ether:petroleum ether (1:1); equal volume of 10% NaCl aqueous solution was added and phase separation was carried out by centrifugation. The total carotenoid content obtained by the developed method was 156.81 ± 1.84 µg/g of dry weight (dw). The extract was saponified and analyzed by HPLC–DAD–MS/MS. Validation parameters included linearity and linear range, as well as limits of detection and quantification (LOD and LOQ). Seven-point analytical curves were constructed for (all-E)-lutein, (all-E)-zeaxanthin, (all-E)-β-cryptoxanthin, (all-E)-α-carotene and (all-E)-β-carotene. Additionally, the method accuracy was determined by recovery of (all-E)-lutein, (all-E)-β-cryptoxanthin and (all-E)-β-carotene st andards spiked in three concentration levels in samples of unripe Valencia orange. Regarding to method performance, the results of repeatability (2.9 to 8.5%) and recovery (82 to 88%) were in agreement with reported values for procedures including all the analysis steps (extraction, saponification and chromatographic separation). The method was applied to analyze the carotenoid composition of saponified extract of Valencia orange pulp. Twenty-five carotenoids were separated and xanthophylls accounted for 98% of the total carotenoid content. The major carotenoids found were (9Z)-violaxanthin (30.3 µg/g dw), (all-E)-lutein (11.5 µg/g dw), (9Z)-antheraxanthin (10.6 µg/g dw), (all-E)-violaxanthin (8.8 µg/g dw), (all-E)-zeaxanthin (7.9 µg/g dw), (9Z or 9'Z)-luteoxanthin (7.8 µg/g dw), (all-E)-luteoxanthin (5.1 µg/g dw) and (all-E)-β-cryptoxanthin (4.2 µg/g dw). Results indicated that the developed method is appropriate for carotenoid analysis in lyophilized orange pulp.

Keywords: *carotenoid extraction, Citrus sinensis, HPLC-DAD-(APCI) MS/MS, new method, xanthophylls*

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D16
CAROTENOID ACCUMULATION DURING RIPENING OF TANGOR CV. MURCOTT AND ORANGE CV. PERA CULTIVATED IN BRAZIL

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Carotenoids are natural pigments responsible for the yellow to red colors of several vegetables and fruits, including citrus. Besides color, carotenoids present important physiological actions, such as provitamin A activity. Moreover, epidemiological studies have suggested a positive correlation between ingestion of vegetables and fruits containing carotenoids and prevention of several chronic-degenerative diseases, such as cancer, cardiovascular diseases and age-related macular degeneration. Therefore, knowledge of the carotenoid composition of food is important. The carotenoid composition of tangor (*Citrus reticulata* X *Citrus sinensis*) cv. Murcott and orange (*Citrus sinensis*) cv. Pera cultivated in Brazil at four ripening stages was characterized. All the samples were cultivated in the city of Capão Bonito (24°0'14" South, 48°20'21" West, São Paulo, Brazil) by the Centro APTA Citros Sylvio Moreira. Fruits were harvested in four months (April, M ay, June, July) corresponding to mature green to full ripe stages. Carotenoids were extracted using the method developed and validated in our laboratory specifically for lyophilized orange pulp. Carotenoid extracts were analyzed by HPLC-DAD-(APCI)MS/MS. In all ripening stages evaluated, orange's main carotenoids were (9Z)-violaxanthin followed by (9Z)-antheraxanthin, while in tangor the major carotenoids were (all-E)-β-cryptoxanthin and (9Z)-violaxanthin. Considerable amounts of (all-E)-violaxanthin, (all-E)-luteoxanthin, (9Z)-luteoxanthin, (all-E)-lutein and (all-E)-zeaxanthin were also found in both varieties. The contents of all the carotenoids increased over ripening; however the most noticeable increments in orange were observed for (9Z)-violaxanthin (8 to 38 µg/g dw) and (9Z)-anteraxanthin (3 to 20 µg/g dw) with total carotenoid content ranging from 49 to 166 µg/g dw. On the other hand, for tangor, remarkable increments in the contents of (all-E)-β-cryptoxanthin (21 to 81 µg/g dw) and (all-E)-β-carotene (1 to 9 µg/g dw) were found, with total carotenoid contents ranging from 106 to 284 µg/g dw. The preferred accumulation of different carotenoids in the full-ripe stage of orange and tangor affects quality since it influence color, but also have important impact on the nutritional value, since higher provitamin A activity is found in tangor cv. Murcott than in orange cv. Pera.

Keywords: *citrus, epoxycarotenoids, HPLC-DAD-(APCI) MS/MS, provitamin A activity, ripening*

Acknowledgement: *São Paulo Research Foundation (FAPESP) (Grants 2013/09804-5 and 2013/07914-8)*

D17
CYANOBACTERIA – A VALUABLE SOURCE OF NATURAL BIOACTIVE COMPOUNDS AND NUTRACEUTICALS

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Cyanobacteria (blue green algae) are morphologically and metabolically diverse, widely distributed, Gram–negative photosynthetic prokaryotes that show several advantages as hosts for biotechnological applications related to food industry. Rapid increase of biomass, ease and low costs of culture maintaining, diversity of metabolism and production of many secondary metabolites, make the cultures of these microorganisms a promising resources for many novel biologically active molecules and compounds of high commercial value. The secondary metabolites, isolated from cyanobacteria, show among others: antibacterial, antifungal, antiviral, anti-inflammatory, antialgal, antiprotozoal, anticancer and antioxidant activities that are of pharmaceutical and agricultural significance. Whereas cyanobacterial pigments: phycobiliproteins and carotenoids, are extensively used respectively in bioindustry and in food industry, where they possess a high commercial value. More over, among so far known phytonutrients formed by cyanobacteria, the greatest interest is focused on flavonoids that are famous for their nutraceutical activity. Interestingly however, the chemical character and quantity of metabolites formed by these photosynthetic microorganisms, can be influenced by changing the conditions of cultivation. Therefore the understanding the nature of the production of bioactive natural compounds, pigments and nutraceuticals by cyanobacteria, is the main goal of our study. In order to recognise the basic relations between the strain of cyanobacteria, the conditions of culture maintenance and the range of metabolites of interest, intentionally simple, "step by step" analytical procedure is still developing, basing on the chemically controlled selection of halophilic and freshwater cyanobacteria, considering the production of mentioned substances. The thin layer chromatography, spectrophotometry, and gas chromatography coupled with mass spectrometry or flame ionization detection, were used to analyse the chemical composition of the extracts from cyanobacterial cells. Obtained results indicated that freshwater strains from Anabaena genus were the most effective producers of cyanobacterial pigments, whereas freshwater Nodularia moravica, Synechocystis aquatilis and halophilic strains: Spirulina platensis, Arthrospira maxima and Arthrospira fusiformis were abundant source of flavonoids.

Keywords: *cyanobacteria, carotenoids, phycobilins, flavonoids, nutraceuticals*

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D18
CAROTENOIDS AND THEIR ESTERS IN MURICI, AN AMAZONIAN FRUIT

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Epidemiological studies have shown a strong association between consumption of fruits and vegetables containing carotenoids and reduced risk of developing non-communicable diseases. Carotenoids are naturally found esterified with different fatty acids in fruits and that is the ingested form of such compounds. The current knowledge about bioaccessibility and bioavailability of carotenoids in foods indicates that the type of consumed carotenoid affects the gastrointestinal fate. For example, carotenoid hydrophobicity affects the efficiency with which carotenoids are transferred into mixed micelles. Thus, the determination of carotenoid esters from fruits is very important. In this study, we identified the profile of carotenoids and their esters in murici (Byrsonima crassifolia), a native fruit from Amazonian region of Brazil. Furthermore, we evaluated the fatty acid profile of murici fruits to support the ester identification. Carotenoids were extracted with a cetone and transferred to petroleum ether/diethyl ether. The non-saponified extract was submitted to a two-step cleanup procedure developed in our laboratory for interferents' removal of carotenoid ester analysis. The procedure comprises the physical separation of lipids under cold conditions followed by open column chromatography (MgO:Diatomaceous earth) for triacylglycerol elimination. Carotenoid extract was analyzed by HPLC–DAD–APCI–MS/MS, in positive ionization mode. Lipids were extracted with chloroform and methanol, converted into fatty acid methyl esters and analyzed by GC–FID. Twenty six carotenoids were separated in the non-saponified extract. Murici carotenoids displayed two different UV-VIS patterns: (i) carotenoids with maximum absorption wavelengths at 445 and 450 nm, represented by xanthophylls with molecular weight of 568 u and (ii) carotenoids with maximum absorption wavelength at 422 nm, including the epoxycarotenoids with molecular weight of 600 u. The first group comprised the main carotenoids of murici fruits. The major carotenoids found were the free xanthophylls (all-E)-lutein (27%) and (all-E)-zeaxanthin (10%), followed by diesters dimyristoyl-(all-E)-lutein (12%), myristoylpalmitoyl-(all-E)-lutein (6%) and lauroylmyristoyl-(all-E)-lutein (5%) and the monoester myristoyl-(all-E)-lutein (7%). Indeed, the fatty acid analysis showed that myristic acid (16:0) was among the major fatty acids of murici. The identification of carotenoid esters is still a challenge, but we successfully identified the main carotenoids in murici fruit.

Keywords: *carotenoid ester, carotenoid identification, fatty acid, HPLC–DAD–APCI–MS/MS, GC–FID*

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D19
A NOVEL TWO-STEP CLEANUP FOR IDENTIFICATION OF CAROTENOID ESTERS BY LC–MS

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Carotenoids are natural pigments recognized as bioactive compounds. They are found esterified with fatty acids in fruits, but this fact is often overlooked on several studies since saponification is routinely done for carotenoid analyses. Due to the high number of possible carotenoid esters and mainly because of the presence of interfering triacylglycerols (TAGs) concomitantly extracted with carotenoids, the identification of carotenoid esters by LC–APCI–MS^n is much more difficult and laborious. In the non-saponified extracts of fatty fruits, TAGs show higher chromatographic signals than the carotenoid esters and some TAGs even have similar fragmentation patterns than those of the carotenoid esters, impairing the correct carotenoid identification. In addition, the high amount of fat also causes high background noise in APCI positive mode ionization. In this sense, we proposed a new two-step cleanup procedure to remove TAGs and other lipids from carotenoid ex tracts from fruits and plant materials. This procedure was first applied to murici (Byrsonyma crassifolia), an Amazonian fruit, orange (Citrus sinensis) cv. Valencia, and Tagetes flowers. The non-saponified carotenoid extract was kept at -18°C for 48 h under N2 atmosphere for physical separation of lipids. After vacuum filtration under cold conditions, the extract was further diluted with petroleum ether followed by open column chromatography (OCC) packed with MgO:Diatomaceous earth (1:1). TAGs were removed by washing the column with petroleum ether and the carotenoids were eluted with more polar solvents. The crude extract and the extracts obtained after physical separation and physical separation plus OCC of each sample were analysed by LC–DAD–APCI–MS^n, in positive ionization mode. The results indicate that most of the TAGs were successfully removed from the murici carotenoid extract. This fact was evidenced by the decrease in the chromatographic co-elution of TAG peaks after the two steps of the cleanup procedure (physical separation plus OCC). In addition, the mass spectra showed less interfering fragments after the cleanup procedure. Indeed, before the cleanup, it was possible to identify only three carotenoid esters and two free xanthophylls in murici, whereas after cleanup we identified twenty carotenoid esters and six carotenoids in the free form. On the other hand, no difference was noticed in Tagetes extracts after the cleanup procedure while the chromatogram from orange extract obtained after the cleanup shows signs of isomerization and loss of compounds. While orange and Tagetes petals presented low fat content, murici contains 15 g.100 g^{-1} of lipids (dry weight), severely affecting the carotenoid ester identification by TAG presence. The new two-step cleanup procedure herein presented can be a useful tool for carotenoid ester identification in fat-containing fruits. This procedure will be applied to the identification of caroteno id esters of mango and papaya.

Keywords: *Carotenoid ester, carotenoid identification, fat-containing fruit, LC–DAD–APCI–MS^n, triacylglycerol*

Acknowledgement: *São Paulo Research Foundation (FAPESP) (Grants 2013/23218-1 and 2013/07914-8)*

D20
HPLC–ESI–QTOF–MS CHARACTERIZATION OF POLYPHENOLS IN STEVIA REBAUDIANA LEAF EXTRACTS OBTAINED BY ADVANCED EXTRACTION TECHNIQUES

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Stevia rebaudiana Bertoni is a perennial shrub native to South America, belonging to the Asteraceae family. The great economic value of Stevia lies in the high content of dietetically valuable sweet-taste compounds of its leaves, which have been used as natural sweeteners for several years. Nowadays, cultivation of Stevia has extended to other regions of the world like North America, Asia and Europe and its extracted glycosides, stevioside and rebaudioside A, are employed as food additives in many countries. The growing economic and scientific interest towards Stevia rebaudiana is not only related to the use of some of its components as non-caloric sweeteners, but also to the therapeutic value and the presence of bioactive compounds in its extracts like amino acids and vitamins, which are being object of investigation by our research group. Some studies revealed that Stevia extracts show antimicrobial and antioxidant activity and many of its phytochemical constituents possess health-promoting properties1. Plant phenolic compounds show a broad spectrum of functional properties. For this reason, researchers and enterprises has focused their attention on the possible use of these phytochemicals in the food industry sector, to provide additional value to common foodstuffs. Moreover, polyphenolic profile is extremely useful for the characterization and classification of plant material according to cultivar and geographical origin. Microwave-assisted extraction (MAE) and pressurized liquid extraction (PLE) were performed in order to extract phenolic compounds from Stevia dried leaves by means of GRAS (Generally recognised as safe) solvents. The total phenolic content of extracts was determined using Folin-Ciocalteu's assay in order to assess the best extraction conditions. The influence of various parameters (i.e. solvent composition, temperature, time) on the extraction yield was evaluated. In addition, high-performance liquid chromatography coupled to electrospray ionization quadropole-time of flight mass spectrometry (HPLC-ESI-QTOF-MS) was used to characterize the bioactive compounds in the richest extracts. A variety of phenolic compounds was identified, mainly phenolic acids and flavonoids, along with the ent-kaurene glycosides responsible for the sweet taste.

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Keywords: *polyphenols, stevia, tandem mass spectrometry*

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D21
A RAPID, LABEL-FREE TECHNOLOGY FOR MICROBIAL IDENTIFICATION FOR FOOD-BORNE PATHOGENS

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Label-free applications for pathogen identification create new possibilities for food safety surveillance. Because of the unique capability of our elastic light scatter (ELS) technology to produce an electronically transferable signature, it would be possible to create a surveillance network focused a variety of food pathogens. ELS methodology for identification of category-B, and selected category-A pathogens has been proven to have high accuracy and reproducibility in organism identification. We have previously demonstrated an ability to identify a variety of known pathogens including Listeria, Salmonella, Vibrio, E. coli, Staph, Enterococcus, Klebsiella, and Yersinia for example. A key feature of this technology is that it is label-free and the colony is not destroyed in the detection process. Elastic light scatter produced from a laser impinging on a colony is collected onto a CCD below the colony and this light scatter produces a fingerprint for that colony. Once that organism is trained within our system, a subsequent identification is now possible directly from an agar plate. This presentation will outline the surveillance model and show data for the above pathogens using the ELS technology and how this innovative approach has many advantages for the microbiology laboratory.

Keywords: *Pathogen detection, Pathogen Identification, Label-free, Classification*

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D22
ANALYSIS OF SELECTED SUBSTANCES WITH ANTIOXIDANT EFFECTS IN MEDICINAL PLANTS

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Oxygen free radicals if produced in excess can cause cell and tissue damage. This damage (a so-called oxidative stress) contributes to premature aging of the organism and the onset and development of a number of serious inflammatory and degenerative diseases. The optimal intake of antioxidants provides a preventive measure against these health risks. Antioxidants are substances capable of reducing the activity of oxygen radicals, decreasing their production and transferring to less reactive or non-reactive states. Fresh vegetables, fruits, and cereals are rich sources of antioxidants. Antioxidants are also found in medicinal plants and plant drugs. This study summarizes results of analyses of selected plant dyes and other antioxidants in some medicinal plants. Parts of the selected medicinal plants (Calendula officinalis, Plantago lanceolata, Hypericum perforatum, Tilia cordata, Sambucus nigra) were analyzed for the content of selected fat-soluble antioxidants. From the samples of medical plants, substances were extracted and analyzed by high-performance liquid chromatography with FLD detection.

Keywords: *Medicinal plants, active substances, antioxidants, vitamins*

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D23
CHROMATOGRAPHIC FINGERPRINT AND BIOLOGICAL ACTIVITY OF POLISH GOLDENROD (SOLIDAGO VIRGAUREA *L.*) HONEY

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Honey quality and authenticity evaluation is an important applied research area with relevant impact on industry and consumer protection. It is estimated that an average of 16,000 tonnes of honey is produced in Poland every year (according to the estimates of Institute of Agricultural and Food Economics). In addition to flavour and nutritional values, therapeutic effect is one of the most valuable qualities of honey. This therapeutic effect not only provides many opportunities for the use of honey as an ingredient in many medicinal products, but also increases the qualities of honey as an element of our diet. Thus, the knowledge of the structures and content of low-molecular organic compounds in various brands of honey might cause better understanding of honey's flavour and nutrient. In this regard, one of the most interesting and relatively new brands of Polish honey is goldenrod honey. It is characterized by non-typical aroma, lemon flavour, high acidity (pH 3.8–4.1) and superior therapeutic properties. The purpose of presented study was evaluation of chemical content of goldenrod honey and construction of chemical profiles based on the HPLC and GC–MS analysis. Additionally, was to determine and identify the markers, i.e. chemical substances characteristic of goldenrod honey, in the class of both phenolic and terpene compounds. Obtained chromatographic data showed that goldenrod honey contains substantial quantities of volatile and phenolic compounds and has a high antioxidant potential. In turn, created the chemical profiles can be used to uniquely identify and assess the quality of honey, and linking these chemical profiles to a therapeutic effect of honey.

Keywords: *goldenrod honey, polyphenolic composition, volatile compounds, chemical profiles,*

D24
COMPARATIVE ANALYTICAL STUDY OF BIOPHENOLS IN EDIBLE OLIVES

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Edible or table or olives are an important component of the Mediterranean diet, which has been linked with protective effects against several diseases such as cardiovascular and neurodegenerative disorders, cancer, inflammation and osteoporosis [1,2]. The composition of edible olives is characterized by the presence of biologically active compounds commonly named biophenols belonging mainly to phenylalcohols (tyrosol, hydroxytyrosol), iridoids (ligstroside, oleuropein), flavonoids (luteolin, apigenin), triterpenoids (maslinic acid) etc. Recently, European Food Safety Authority (EFSA) published its scientific opinion declaring that the daily consumption of at least 5 mg of hydroxytyrosol and/or its derivatives (e.g. oleuropein complex and tyrosol) protects LDL particles from oxidative damage [3]. However, there is a strong association between the qualitative and quantitative profile of polyphenols, and olive tree variety, environmental conditions (such as so il composition, climate), storage time while debittering processing methods has a significant impact as well. The aim of this work is to compare and further investigate the biophenols content of table olives prepared using two different debittering processing methods i.e. physical or organic (addition of NaCl) and chemical (treatment with NaOH) method. Even though both methods are widely employed for the debittering of olives, there are limited qualitative and quantitative data available. Furthermore, fraud incidences usually occurred since there are no established methods available defining the processing method. Thus, in the current work several analytical techniques such as HPLC–DAD, HPTLC, LC–HRMS, and NMR (1 & 2D) in a successive and complementary way were incorporated for the analysis of the different olive samples while much attention was given to the extraction procedure and the determination of biophenols total yield. Furthermore, the brine (debittering water) of all samples under study was also analyzed supporting the detected variation. According to our data significant di fferences were observed between the two different debittered olives as well as the corresponding waste water while a detailed chemical profile was also determined.

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Keywords: *edible olives, polyphenols, debittering process, LC–MS, NMR*

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D25
INVESTIGATION OF ANTIOXIDANTS BY POTENTIOMETRIC METHOD USING COMPLEXES OF METAL

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Free radicals are a necessary component of many biochemical processes. However, under the influence of unfavorable factors such as environmental degradation, poor quality food intake, UV radiation and others. Observed enhancement of free radical oxidation (oxidative stress). Free-radical oxidation is a major pathogenetic mechanism of a huge number of diseases, such as diseases of the central nervous system, cardiovascular diseases, diseases of the reproductive system, eye diseases and the aging process in general. The harmful effects of oxidative stress can be reduced by the consumption of natural antioxidants. The most promising are the products of vegetable origin, rich in polyphenols, vitamins, carotenoids and others. Polyphenol compounds and vitamins with antioxidant properties present in large quantities in fruits, vegetables, tea, wine, etc. and can also act as radical traps, and to interrupt the chain reaction of radical formation. This paper proposes a method of potentiometric investigation of antioxidants, based on the use of the oxidized form of the metal complex compound composed of a model oxidizer. Basic requirements oxidizer: electrochemical reversibility, high value of the conditional stability constants in the range of pH close to physiological. Measurement of potential is carried out after passing through a chemical reaction between a test sample and oxidizer, and subsequent addition of the oxidizer or the sample. Polyphenolic antioxidants studied nature with different amounts of antioxidant OH-groups. Also investigated the total antioxidant activity of a number of food products made from vegetable juices of fruit and vegetables both fresh and industrial manufacturing. We studied some teas. Elevated levels of antioxidants observed in foods rich in water-soluble vitamins (including vitamin C) and having a high content of polyphenols. Proposed potentiometric method for the determination of antioxidants is simple, requires no costly reagents and takes a short time. It can be used to assess the quality of finished products, as well as for control of raw materials in the production process and during storage.

Keywords: *antioxidants, electrochemistry, metal complex*

D26
ANTIOXIDANT CAPACITY AND PHENOLIC CONTENT FROM FRUIT AND VEGETABLES FLOURS IN GLUTEN-FREE COOKIES PROCESS PRODUCTION

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Celiac disease is an autoimmune disorder caused by intolerance to gluten, which is found in wheat and similar proteins such as barley, rye and oats. This disorder is characterized by the inability to digest a protein fraction, classified as gluten. A person with this syndrome, which appears usually in early age, cannot consume a number of cereal products, particularly pasta and bread derivatives, and the acceptable treatment is a strict adherence to a 100% gluten-free diet for life. The only effective treatment for celiac disease is to maintain a strict gluten-free diet. Replacing the cereals with gluten, mainly for rice flour, it can be obtained a satisfactory result, but most of those products are tasteless, with pour mouth fell and with low nutrient content. The inclusion of fruits and vegatables can be an interesting choice, they are an important source of food fibres, minerals and vitamins, and are also recommended by health organizations and dieticians .The antioxidant term is used to describe the cell protective function against the damaging effects of free radicals. Some nutrients, naturally occurring or added in foods have antioxidant properties. These combat the so-called free radicals, which are unstable and reactive molecules. The objective of this study was the enrichment of gluten-free cookies with antioxidants and polyphenols from different fruits and vegetables. The enrichment of cookies was done with the addition of strawberry, banana, mango and beet flours obtained by lyophilized process. In the present study 5% of flour from different fruits and vegetables were added to a gluten-free cookie formulation. The content of polyphenols and related antioxidant activities of raw cookie dough and baked cookie were compared. The amount of antioxidants was calculated by the DPPH method, based on the capture of DPPH (1,1-Diphenyl-2-picryl-hydrazyl) by antioxidants, producing a decrease in absorbance at 515 nm, the results are expressed in EC50 (sample mass / DPPH mass). The phenolic content of the obtained extracts was estimated by a colorimetric method using the Folin-Ciocalteus's solution. The obtained results profiles allowed the comparison of the different groups of fruits and vegetables effect on gluten-free cookies. The results showed that strawberry flour cookies are richer in polyphenols content than with the other fruits, containing 50% more than beet cookies, 75% more than mango cookies and 90% more than banana cookies. Results also indicated that the cooking process doesn't affect the polyphenols content. Regarding antioxidant content, it was observed that strawberry cookies flour has the highest antioxidant activity, followed by beet, mango and banana flours. It is therefore concluded that the addition of fruit powder is a way of enriching gluten-free cookies. Also a mixture of these fruits and vegetables could be recommended for consumption.

Keywords: *gluten-free, antioxidant activity, phenolic content*

D27
SIMULTANEOUS DETERMINATION OF LYCOPENE, BETA CAROTENE AND VITAMINS BY ON-LINE COLUMN FOCUSING FOLLOWED BY REVERSE PHASE LIQUID CHROMATOGRAPHY AND UV DETECTION

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Vitamins and carotenes represent important dietary components essential for health, because of their involvement in vital metabolic processes, the potential ability to decrease the risk for coronary heart disease, and the possible role in prevention of cancer and sight disorders. Therefore, their content in food commodities can be used as an index of the health-related quality of products. Established methods for analysis of vitamins include the chromatographic ones that are typically designed for classes of compounds, i.e. water- and fat-soluble vitamins, based on their different solubility properties and chemical structures, ranging from small organic acids to large conjugated complexes. Water-soluble vitamins are usually separated by reverse phase Liquid Chromatography (LC) with aqueous mobile phases, while for fat-soluble vitamins organic solvent mobile phases both reversed and normal-phase LC are used. The simultaneous determination of water and fat sol uble vitamins, in addition to highly lipophilic compounds, such as carotenes, increases the complexity of an analytical method, including sample processing and storage, as well as the choice of proper solubilisation solvents, which must be in tune with the chromatographic eluents. Moreover, the heterogeneity of food matrices and the potential degradation of these classes of compounds during the analysis make their quantitative determination even more demanding. Therefore, although several LC methods have been reported [1,2], the simultaneous determination of vitamins and carotenes usually suffer from incomplete chromatographic resolutions and low throughput. In this study, an improved LC method coupled to a multiple wavelength UV detection is described for the determination of B group vitamins (B1, B2, B3, B6 and B9), ascorbic acid (vitamin C), retinol (vitamin A), tocopherol (vitamin E), phylloquinone (vitamin K1), lycopene, β-carotene, and β-apo-80-carotenal, used as an internal standard. Chromatographic separations have been performed by an innovative core-shell C18 column packed with 2.6 μm particles; flow rates and gradient elution programs have been carefully optimized for the total resolution of all the target analytes. An on-line focusing step was required for column head trapping of carotenes and fat-soluble vitamins, before the chromatographic run starting from an aqueous mobile phase, necessary for the elution of scarcely retained water-soluble vitamins. Under the optimized chromatography conditions, an efficient separation has been obtained in less than 25 minutes. Rapid and simple procedures for the extraction of c arotenes and vitamins have been established; hence the potential of the proposed method has been confirmed by the analysis of tomatoes and fresh fruits.

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Keywords: *vitamins, carotenes, on-line column focusing, high performance liquid chromatography, UV detection*

D28
ANALYSIS OF CALYSTEGINES IN FOOD PRODUCTION CHAIN

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Calystegines are toxic secondary metabolites naturally occurring in plant of *Solanaceae*, family including important food crops such as potatoes (*Solanum tuberosum*), in which the most important are calystegines A3, B2, and B4. Calystegines in potato tubers and/or potato products can be affected by many factors such as, stress factors during plants growth, mechanical damage during harvest or inappropriate storage condition. To assess consumers´ health hazard, European Food Safety Authority (EFSA) requires more occurrence and toxicity data.
In our study, the changes in calystegine levels (A3, B2, B4) were monitored 21 Swedish potato tuber varieties. Tubers were subjected to (i) mechanical wounding, (ii) light exposure and (iii) elevated temperature. Ultra-High Performance Liquid Chromatography on HILIC separation column coupled with tandem Mass Spectrometer (U-HPLC-QTRAP–MS/MS) was used for the determination of these compounds. Significant changes in calystegine content were noted only for calystegine B4. However, the total calystegine levels were not affected by wounding or light exposure.
Changes in content and representation of calystegines (A3, B2 and B4) in fresh and stored potato tubers and French fries was also monitored. Calystegine content may be influenced during the processing of potato tubers.

Keywords: *U-HPLC–MS/MS, calystegines, potato tubers, EFSA*

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D29
ANALYTICAL APPROACHES FOR CHARACTERIZATION AND QUANTITATION OF HYDROLYSED COLLAGEN, AS A COMPONENT OF DIETARY SUPPLEMENTS

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Hydrolyzed collagen is one of the most widely used dietary supplements component taken for the joint nutrition. Because the biological availability of the hydrolyzed collagen significantly differs according to the length of the polymer, there is a need to develop a robust and reliable analytical approach for control of its molecular-weight character and quality.
Within our study, the method based on the size-exclusion chromatography separation, together with the diode array detection, was developed and critically assessed in comparison with the 'classical' approach enabling the total hydrolysis, and calculation of collagen based on the hydroxyproline content. As concerns the size-exclusion high-performance liquid chromatography, the Yarra™ 3µ-SEC 2000 (Phenomenex) column was selected as an optimal one with regard to its separation range. As a mobile phase, 50 mM phosphate buffer with 300 mM sodium chloride at pH 6.8 was used. The detection of hydrolyzed proteins was realized with the 220 nm wavelength, referring to the absorption of the peptide bonds. For validation of this SEC–LC–UV method, repeatability expressed as relative standard deviations (RSDs, %) was determined by using of the internal reference material of hydrolyzed collagen-based dietary supplement. The RSD value for the food supplements was 5%. For calculation of molecular weight of proteins / peptides contained in analyzed samples, calibration curve relating the retention time and molecular weight (protein standards size 2,844–669,000 Da) was established. The method was then applied to the analysis of 12 food supplements.
As already mentioned above, to verify the accuracy of the SEC–LC–UV method, we optimized a method for hydroxyproline quantification in the total food supplement hydrolyzate. The analysis of free hydroxyproline (together with other free amino acids) was conducted by using ultra-high pressure liquid chromatography (HILIC–based chromatography with Atlantis HILIC Silica 3 µm (Waters) column) coupled with high resolution mass spectrometer with orbitrap mass analyzer (Exactive, Thermo Scientific). Both of these methods provided similar results of hydrolysed collagen.

Keywords: *collagen, food supplement, size exclusion chromatography, hydroxyproline*

Acknowledgement: *The financial support of the Technology Agency of the Czech Republic, project No TE01020080.*

D30
ANALYSIS OF CAROTENOIDS IN MARIGOLD FLOWERS USING HPLC–DAD

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Marigold flowers (*Calendula officinalis* and *Tagetes species*) have been known as a rich source of various biologically active compounds among which carotenoids play the major role. Carotenoids act as colorants and are one of the groups of phytochemicals responsible for the petals color. They also exhibit many positive health effects on human body. This leads to a still increasing demand for marigold flowers extracts in the food and medical industry. In this work we have reported the study on the carotenoids present in marigold flowers. The attention was focused on the optimization of the method for carotenoid determination. Several extraction techniques were carried out and samples were analyzed under different analytical conditions using HPLC coupled with the diode array detector (DAD). The influence of various factors as the effect of plant variety, the harvest season or the technique of preservation on the major carotenoids levels in marigold flowers were also examined. Based on the experimental data a robust and selective method for the determination of carotenoids in marigold flowers has been developed. In Calendula flowers, the content of monitored carotenoids ranged from 82 to 1472 mg/kg on the dry weight of flowers for lutein and from 5 to 1300 mg/kg on the dry weight of flowers for β-carotene, depending mostly on the harvest season and post-harvesting processing. In Tagetes flowers, the levels of lutein were much higher than in Calendula flowers, ranging from 3400 to almost 26000 mg/kg on the dry weight of flowers, while the amounts of β-carotene were on an insignificant level.

Keywords: *Calendula officinalis, Tagetes species, lutein, β-carotene, HPLC–DAD*

Acknowledgement: *This study was carried out with financial support from the Technology Agency of the Czech Republic project BIORAF No. TE01020080 and specific university research (no. 20/2015).*

D31
ANALYSIS OF FLAVONOIDS IN MARIGOLD FLOWERS USING UHPLC–ORBITRAP MS

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Marigold is one of the most important ornamental plants in the world. The flowers of this plant have found their applications in food and traditional medicine since ancient times due to their edible and medicinal properties. These flowers occur in yellow to golden orange coloration depending on the concentration of color pigments. The phytochemicals responsible to color pigmentation in flowers include carotenoids, flavonoids, anthocyanins, etc. These compounds are also biologically active compounds beneficial for health, thus expanding the use of marigold flowers in food and pharma industry further. In our study, we analyzed two commonly known genus of marigold: Calendula officinalis and Tagetes species that belong to Asteraceae family; for their flavonoid content. The extracts were analyzed by UHPLC–Orbitrap MS using ESI± ionization mode. The detection of compounds was performed by high resolution MS technique based on the mass to charge ratio of the ion obtained in negative ionization mode as it yielded better results than positive ion mode. Identified flavonoids were semi-quantified using reference standards (quercetin and rutin) to obtain tentative concentrations of detected individual flavonoids. The detected flavonoids included calendoflavoside or isorhamnetin-3-O-rutinoside, calendobioflavoside, isorhamnetin galactoside, etc. in Calendula flowers and patuletin or quercetagetin methyl ether, myricetin or quercetagetin, patuletrin, etc. in Tagetes flowers. The concentration of flavonoids ranged from 3 to 75 g/kg on the dry weight of Calendula flowers while from 7 to 20.3 g/kg on the dry weight of Tagetes flowers.

Keywords: *Calendula officinalis, Tagetes species, flavonoids, UHPLC, Orbitrap–MS*

Acknowledgement: *This study was carried out with financial support from Technology Agency of the Czech Republic, project BIORAF No. TE01020080 and specific university research (MSMT no. 20/2015).*

D32
APPLICATION OF HIGH RESOLUTION MASS SPECTROMETRY FOR ANALYSIS OF GALACTOLIPIDS IN ROSE HIPS

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Rose hips are a fruit of Rosa species from Rosaceae family and they are well known to possess beneficial biological effect on human health such as anti-inflammatory effect, anti-oxidation and anti-tumor activity. And that is due to many groups of biologically active compounds like phytosterols, flavonoids, carotenoids or vitamin C. Rose hips have become a very important material for production of food supplements. In present, there are several rose hip powder based food supplements on European market and they are designated mainly for joint care. It has been suggested, that the group of compounds responsible for some beneficial effect of rose hips and food supplement based on rose hips are galactolipids. Clinical studies indeed suggest that galactolipids may help patients suffering from inflammatory diseases like osteoarthritis in a way of pain reduction, symptom alleviation and prevention in healthy patients. Basically galactolipids are polar lipids, diacylglycerols with one or two bonded units of galactose. According to the number of bound molecules of galactose, they can be divided into monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG). Galactolipids may also differ in chain length and double bonds number of fatty acids bonded in galactolipid molecule. Based on these premises, we can expect that there are many combinations of existing galactolipids. The method of extraction and identification of galactolipids have been developed and validated with use of ultra performance liquid chromatography coulpled with high-resolution tandem mass spectrometry (UPLC–MS/MS, Synapt G2, Waters). Both normal and reverse phase columns were tested, normal phase column was tested for separation of galactolipids according to number of bonded galactose units and reverse phase for separation based on bonded fatty acid. This method was applied to samples of rose hips and food supplements based on rose hip powder. So far 26 different galactolipids were identified in analyzed samples according to their MS/MS spectra, 14 MGDGs and 12 DGDGs. Relative abundance data suggest that there are 2 major galactolipids MGDG and DGDG with two bonded α-linolenic acids. Together, these two galactolipids represent more than a half of all detected galactolipids.

Keywords: *rose hips, galactolipids, LC–MS/MS*

Acknowledgement: *This research was carried out within the project MSMT COST LD14092 and specific university research MSMT no. 20/2015 (supported by the Ministry of Education of the Czech Republic)*

D33
DETERMINATION OF MAJOR PHYTOCANNABINOIDS EMPLOYING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION TANDEM MASS SPECTROMETRY TECHNIQUE

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Cannabinoids belong to a group of chemicals naturally occurring in Cannabis plants including three main strains, *Cannabis sativa, Cannabis indica,* and *Cannabis ruderali*.. Cannabinoids, pattern of which fairly differs among these plants, elicit various biological effects in living organisms: while some of them, represented mainly by Δ9-tetrahydrocannabinol (THC), are psychotropic, they may also act as antioxidant and neuro-protectants, cannabidiol (CBD) being probably the most popular compound. Up to now, almost 100 of phytocannabinoids, synthetic cannabinoids, and also endocannabinoids have been described. THC and CBD may originate from their precursors in biosynthetic pathway, tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), respectively, as the result of photo- and/or thermodegradation. In any case, effective analytical control of cannabinoids levels in respective plants and products is of a high concern. Currently, liquid chromatography separation coupled to mass spectrometric detection is a preferred technique used for cannabinoids analysis since it provides sufficiently low limits of quantification (LOQ) in a variety of matrices. One of the analytical challenges encountered in phytocannabinoids analysis is a very wide range (even several orders of magnitude) of concentrations at which they occur in analyzed samples. This makes quantification process rather difficult, specifically when HR mass analyzers with a relatively narrow dynamic range (TOF, Orbitrap) are employed for detection. Within this study concerned simultaneous analysis of 7 phytocannabinoids, we focused on overcoming this problem by various settings of Q-Orbitrap analyzer combining different MS experiments in order to ensure sufficient sensitivity for low-abundant analytes and prevent from detector saturation in case of high-abundant analytes. From the variety of tested parameters including fragmentation, period, and number of ions to be injected into the orbital ion trap, we selected targeted single reaction monitoring (tSIM) with fast injection to orbitrap and product reaction monitoring (PRM) with slow injection to orbitrap for low- and high-abundant analytes, respectively.

Keywords: *Natural cannabinoids, UPLC–HRMS, quantification*

Acknowledgement: *The financial support of the Technology Agency of the Czech Republic, project No TA04010331.*

D34
POLAR γ-ORYZANOL, A NEW CLASS OF γ-ORYZANOL IN RBO: IDENTIFICATION AND MONITORING OF QUALITATIVE AND QUANTITATIVE ALTERNATIONS THROUGHOUT THE RICE MILLING PROCESS

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Rice Bran Oil (RBO) is a commonly used cooking oil in Asia, which is obtained from bran, a by-product of the rice milling process. In comparison to other vegetable oils, RBO contains higher amount of γ-oryzanol (γOR). γOR is a mixture of more than 12 ferulic acid esters of phytosterols & triterpene alcohols and has been reported to display an important pharmacological profile i.e. antidiabetic, antihyperlipidemic and anti-inflammatory activity [1]. Recently, a new chemical entity similar to γOR, called "polar" γ-oryzanol (PγOR) was identified in RBO. It is comprised of hydroxylated derivatives of γOR components which purification as well as unambiguous structural elucidation have not been achieved so far [2]. The aim of the present study is the determination and semi-quantification of both γOR and PγOR components in rice bran and rice husk of different varieties (Gladio and Ronaldo) obtained throughout the various steps of the milling process, with or without the parboiling technique (PB) via HPLC–DAD and UHPLC–HRMS/MS techniques. Initially, different extraction methods were assayed. Ultrasonic extraction using EtOAc was the optimum being specific for both γOR and PγOR with yields up to 25% (*w/w*). Additionally, a targeted HPLC–UV method was developed for the separation and quantification thereof. 12 different samples were analyzed and measured concentrations were in the range of 1 mg/g and 10 mg/g for γOR and PγOR respectively. Highest concentrations of γOR and PγOR were found in the extracts from the first whitening step. In Gladio parboiled γOR and PγOR levels were generally maintained during the process in contrast to other preparations. The second part of the study includes the isolation, via FCPC & preparative HPLC and the structural elucidation of the individual constituents of both γOR and PγOR using LC–HRMSn and NMR techniques. For this purpose one step isolation of PγOR was performed via FCPC. Preparative HPLC was used for further purification of the rich in PγOR FCPC fractions. This is the first time that detailed information regarding the PγOR content in different RBOs is reported as well as the thorough characterization of its constituents. Moreover, purification thereof is ongoing as well as evaluation of PγOR and γOR biological properties.

[3] Lemus, C., et al., γ-Oryzanol. An Attractive Bioactive Component from Rice Bran, in Wheat and Rice in Disease Prevention and Health. 2014. p. 409-430.
[4] Angelis, A., et al., One-step isolation of gamma-oryzanol from rice bran oil by non-aqueous hydrostatic countercurrent chromatography. J Sep Sci, 2011. 34(18): p. 2528-37.

Keywords: *polar γ-Oryzanol, rice bran oil, ferulic ester, FCPC, HPLC-DAD, UHLC-HRMS, NMR*

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FLAVOURS
AND
ODOURS

(E1 – E16)

E1
VOLATILE COMPOUNDS FORMING THE MORAVIAN FRUIT SPIRITS AROMA

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Fruit spirits are currently considered as one of the typical alcoholic beverages of Moravia and gastronomic heritage of Moravian culture. The first mentions of the production of fruit spirits in the manor distilleries appear in the 16th century, and planting fruit trees, creation of gardens and fruit nurseries were supported by the several monarchs during centuries. Production of fruit spirit, especially the most known spirit from plums (often called plum brandy), is not widespread in the world. Strong tradition of its production is in lands on Balkan and in Central Europe, particularly in Moravian region (Czech Republic). Fruit as sources for production of fruit spirit in Czech Republic are different; distillates are produced from stone fruit, pome fruit and various kinds of berries. Therefore, the aromas of fruit spirits are very diverse. In this work, aroma profiles of Moravian fruit spirits from stone fruit and pome fruit were investigated. Headspace–solid phase microextraction (HS–SPME) connected with gas chromatography–mass spectrometry (GC–MS) was used for analysis of 20 samples of fruit Moravian spirits. Central composite design of experiment was used for evaluation of optimal extraction parameters. Identification of individual compounds of spirit aroma was performed by comparing mass spectra of analyzed compounds with mass spectra from libraries, and by comparing of retention indices.

Keywords: *fruit spirits, aromaprofile, volatile compounds, headspace SPME, GC–MS*

E2
EVALUATION OF SOLID PHASE MICROEXTRACTION METHOD USING TWO TEMPERATURE SORPTION APPROACH FOR AROMAPROFILE ANALYSIS OF BARLEY AND WHEAT MALTS

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The main aim of this study was the evaluation of a modern sampling approach based on the use of two sorption temperatures in the one headspace solid-phase microextraction (HS–SPME) procedure and its applicability to the analysis of aromaprofile of barley and wheat malt samples. For separation and identification of target compounds the method of gas chromatography–mass spectrometry (GC–MS) together with comparison of retention indices of individual compounds with those found in spectral libraries and/or available on-line databases were used. This modern sorption strategy based on using more sorption temperatures in one procedure, is aiming at extracting a significantly higher number of compounds with wider range of volatilities comparing to conventions sorption strategy using only one sorption temperature. As an analytical tool, experimental designs were used for the method optimisation. First of all, the most significant factors affecting the whole procedure were evaluated by the Plackett–Burman design. After that, the final experimental conditions were established by five-level orthogonal central composite design followed by response surface modelling. During the optimisation experiments it was observed that higher temperature improves the analyte response in the case of less volatile compounds whereas lower temperature is more advantageous in the case of more volatile analytes. The proposed method presents a very good compromise of conditions under which the right combination of sorption temperatures could achieve a much better extraction enabling identification of more volatile and semi-volatile compounds in obtained chromatograms. The proposed method was compared to conventional HS–SPME using single temperature sorption approach. It was confirmed that the presented method provides a better results considering the response in terms of both the total peak areas and mainly the number of identified compounds. The use of two temperature sorption strategy for HS–SPME proved to be a very good tool for screening of volatile compounds in barley and wheat malts with a wide range of volatilities.

Keywords: *SPME, two temperature sorption strategy, aromaprofile, barley and wheat malt, GC–MS*

E3
ANALYSIS OF PHENOLIC ANTIOXIDANTS IN EDIBLE OIL/SHORTENING USING A UHPLC SYSTEM WITH PDA DETECTION

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Phenolic antioxidants are commonly used in food to prevent the oxidation of oils. Oxidized oil and fats cause foul odor and rancidity in food products, which is a major cause for concern to the food industry. This application note presents a UHPLC method for the analysis of the ten most common phenolic antioxidants that may be found in such products. The application was carried out with minor modifications to the AOAC Official Method 983.1 [1.] A 2.7-µm SPP (superficially porous particle) C18 column was used, allowing one to achieve very high throughput at a back-pressure considerably lower than that for UHPLC columns. Method conditions and performance data, including linearity and repeatability, are presented. This method was then applied to a commercial vegetable shortening product.

[1] Official Methods of Analysis, Method 983.15, Association of Official Analytical Chemists (AOAC), Arlington, VA USA

Keywords: *phenolic antioxidants, oxidation of oils, uHPLC*

E4
A MICROWAVE ASSISTED EXTRACTION METHOD APPLIED FOR THE PRODUCTION OF FLAVOURED EXTRA VIRGIN OLIVE OIL WITH AROMATIC PLANTS

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The olive tree (*Olea europea*) is widely cultivated for the production of both olive oil and table olives and is of significant economic importance. Greece is the third largest olive oil producer worldwide. Extra virgin olive oil is unique amongst other vegetable oils because of its increased concentration in characteristic secondary metabolites. Many studies have reported that olive oil has a role in the prevention of coronary heart diseases. Since antiquity aromatic plants have been used in food flavouring, pharmaceutical and perfumery. Medicinal and aromatic plants (MAPs), are also used in the production of flavoured olive oils. Production and consumption of flavoured olive oils are increasing; market studies have demonstrated that consumers, especially from areas outside the Mediterranean area, are interested in this type of product. Flavoured olive oils are usually produced using one of the following methods: (i) macerating the aromatic plants in olive oils and (ii) adding the flavours to the olive oil. In the present study the medicinal and aromatic plant material (oregano, thyme, rosemary, basil, garlic and lemon peel), was directly put into the extra virgin olive oil and a microwave assisted extraction was applied to the mixture in order to accelerate diffusion of the volatile compounds into the olive oil. A microwave industrial scale reactor was used. The apparatus consists of four magnetrons (4 × 1500 W, 2450 MHz) with a maximum power of 6000 W delivered in 500 W increments. The stainless steel microwave cavity has a capacity of 150 L and contains a removable, rotating PTFE drum that allows up to 75 L of plant material to be loaded. Rotation ensures a homogeneous microwave distribution to the material inside the drum. In relation with the plant material the whole procedure lasts from 45 min (oregano) to 50 min (basil). The volatiles of the flavoured olive oils were analyzed by HS-SPME/GC/MS. A 50/30 µm DVB/CAR/PDMS fiber was used to extract head space volatiles. Four grams of flavoured olive oil was put in a 20 mL vial. The vial was maintained in a water bath at 60°C during the whole procedure. Equilibration time was set at 20 min, followed by 40 min sampling time. Tentative identification of the compounds was based on the comparison of their mass spectra with those of the literature and library data of the GC/MS system. In particular, carvacrol, thymol, eucalyptol, linalool, 3-Vinyl-[4H]-1,2-dithiin and limonene were detected in the oregano, thyme, rosemary, basil, garlic and lemon flavoured olive oils, respectively. The main advantage of this new technique is that the essential oil contained in the aromatic plants was directly extracted into the olive oil without any intermediate stage. This process leaded to a flavoured olive oil in few minutes, which can be opposed to the several hours required in conventional maceration process.

Keywords: *flavoured oils, extra virgin olive oil, medicinal and aromatic plants, microwave assisted extraction, HS-SPME/GC-MS*

E5
DETERMINATION OF ESTRAGOLE LEVELS IN FENNEL TEAS BY GC/MS AND ASSESSMENT OF DIETARY EXPOSURE

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Herbal teas based on fennel (*Foeniculum vulgare Mill.*) have a long time history of use for the symptomatic treatment of gastrointestinal and respiratory disorders in Europe and Asia. Despite the beneficial effects of fennel, there is reasonable suspicion that the main compound estragole has the potential to be harmful for human health. The purpose of this study was to determine levels of estragole in different fennel teas available on the Austrian market in order to assess the associated dietary exposure of different population groups such as children, women and men. Additionally, the MOE approach was applied to assess the health risk associated with the consumption of fennel tea. In total, fennel tea products of 42 different brands (tea bags, n = 20, dry fennel seeds, n = 19, instant teas, n = 3) were collected on the Austrian market. Fennel tea infusions were prepared as recommended on the respective label of the product. Estragole was isolated from tea samples by Liquid Liquid Extraction technique. Subsequently, a modified gas chromatography/mass spectrometry (GC/MS) method published by the Federal Office of Consumer Protection and Food Safety (BVL, 2004) was applied. The results show an average estragole content of 565.1 µg kg^{-1} in infusions prepared from tea bags and 639.3 µg kg^{-1} in infusion prepared from dry fennel seeds. Teas specifically marketed for infants comprise a rather heterogeneous group consisting of instant teas, in which estragole levels were below the LOQ (10 µg kg^{-1}) and tea bags with average contents of 78.0 to 415.6 µg kg^{-1}. The estimated daily exposure from consumption of fennel teas prepared from tea bags was 0.41–4.02 µg kg^{-1} d^{-1}, 0.52–5.13 µg kg^{-1} d^{-1}, and 0.24–2.34 µg kg^{-1} d^{-1} for children, women and men, respectively. For teas prepared from fennel seeds daily intakes of 0.25–5.04 µg kg^{-1} d^{-1} (children), 0.32–6.42 µg kg^{-1} d^{-1} (women), and 0.15–2.93 µg kg^{-1} d^{-1} (men) were estimated. Estimated daily intakes of estragole assuming the consumption of fennel tea prepared from teas specifically marketed for infants were in the range of 0.5–20.78 µg kg^{-1} d^{-1} assuming a bodyweight (bw) of 5 kg. Corresponding MOE values were in the range of 400–66,000. Despite calculated MOE values are above 10,000 for nearly half of the fennel teas analysed within this study, there are still MOEs below this value, indicating a potential risk for human health and a priority for risk management. However, it must be emphasised that in the present study MOE values were calculated assuming a lifetime exposure while fennel teas are generally consumed only for short periods of gastrointestinal disorders. Hence, estimated daily intakes might overestimate the potential risk for human health.

Keywords: *estragole, fennel tea, GC/MS*

E6
CHARACTERIZATION OF CYANOBACTERIAL STRAINS REGARDING THEIR PROFILE OF VOLATILE ORGANIC COMPOUNDS

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Microalgal biomass can be utilized for production of functional food and feed products, but certain species of microalgae and cyanobacteria are known to produce various compounds causing off-flavour. In this work, we investigated selected cyanobacterial strains of Spirulina, Anabaena and Nostoc genera originating from Serbia, with the aim of determining the chemical profile of volatile organic compounds produced by these organisms. Additionally, the influence of nitrogen concentration during growth on the production of these compounds was investigated for the Nostoc and Anabaena strains. In addition, multivariate technique, namely principal component analysis (PCA) was used for making distinction among different microalgal strains. The results show that the main volatile compounds in these species belong to medium chain length alkanes, but other odorous compounds such as 2-methyl-isoborneol (0.51 –4.48%), 2-pentylfuran (0.72–8.98%), β-cyclocitral (0.00–1.17%) and β-ionone (1.15–2.72%) were also detected in the samples. Addition of nitrogen to growth medium was shown to negatively affect the production of 2-methyl-isoborneol. Presence of geosmin, which is very important component of odour, was not detected in any of the tested samples, which may be explained by the presence of relatively high concentration of copper ions in the growth medium. Principal component analysis (PCA) has shown good separation of analyzed samples regarding the type and concentration of their volatile compounds and samples of Spirulina were characterized as notably different from other cyanobacterial strains with regard to their high content of alkanes. It can also be concluded that growing conditions can have a significant impact on production of volatile and odorous compounds in cyanobacteria, and altering these conditions may be useful in obtaining cyanobacterial biomass with favorable sensory properties for potential use in formu lation of food and feed products.

Keywords: *microalgae, cyanobacteria, volatile compounds, odour*

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E7
QUALITY CONTROL OF STRAWBERRY FLAVOURED SYRUPS BASED ON ANALYSIS OF VOLATILE COMPOUNDS

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Quality and authenticity assessment of flavoured foods is a specific interest in the food industry. Flavours (aromas) are widely used food additives which improve food odour. They are applied in different types of food, e.g. confectionery, beverages, dairy products and syrups. Flavours (aromas) can be defined as a natural flavouring, natur-identical flavouring or artificial flavouring substances. The aim of this work was to evaluate the quality and origin of aromas as well as their addition to strawberry flavoured syrups based on determination of volatiles profile by solid-phase microextraction coupled with enantioselective-gas chromatography-mass spectrometry (SPME–Es–GC/MS). The following set of products was analyzed: commercially available strawberry aromas, authentic fresh strawberries and strawberry syrups purchased from the market. The results obtained have been statistically processed and compared with the results of gas chromatography-olfactometry (GC–O) and sensory analysis. For a number of samples of syrups we were able to detect addition of synthetic strawberry aroma based on sensorial aroma evaluation or analysis of target compounds. However chemometric evaluation of the entire fingerprint was proved as a suitable tool for detection of synthetic aroma addition to syrups based on natural strawberry juice.

Keywords: *strawberry aroma, syrup, quality, volatile compounds, SPME–Es–GC/MS*

Acknowledgement: *The authors express thanks to the companies Fontea a.s., Poděbradka a.s. and Döhler cz s.r.o. for the provided samples.*

E8
ROLE OF MICROORGANISMS IN THE PRODUCTION OF AROMA COMPOUNDS DURING COCOA FERMENTATION

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The aromatic quality of the chocolate depends mainly of the fermentation step of cocoa beans. The fermentation step contributes to the development of aroma precursors from biochemical reactions realized by microorganisms. Few studies have shown the impact of microorganisms in the development of volatile compounds during cocoa fermentation. The aim of our study was to determine the volatile compounds origin detected in spontaneous fermented cocoa beans. This was done through studying the aromatic profiles of some representative microbial strains isolated from Ivoirian fermented cocoa beans. Monoculture and coculture of cocoa beans have been performed using *Acetobacter pasteurianus* (Acetic Acid Bacterium), *Lactobacillus fermentum* (Lactic Acid Bacterium), *Pichia manshurica* and *Pichia kudriavzevii* (yeasts). Volatile compounds were identified by Solid Phase Micro-extraction coupled to Gas Chromatography and Mass Spectrometry (SPME–GC–MS) from fresh, spontaneous and artificial fermented beans. The results showed that the bacteria mainly produce acids, ketones and aldehydes on the one hand, and yeasts elaborate mainly alcohols and esters on the other hand. Esters gave a floral and fruity aroma to cocoa. Thus yeasts had more impact on aroma quality.

Keywords: *aroma compound, cocoa, acetic and lactic acid bacteria, yeast, SPME–GC–MS*

E9
ANALYSIS OF BOAR TAINT CAUSING COMPOUNDS BY MEANS OF GC/MS

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The surgical castration of piglets without anesthesia is a common method in the European Union. Castration serves to avoid the development of undesirable odors in male pigs, which are formed primarily in the testicles [1]. Responsible for the boar taint odor are the pheromone androstenone and several protein degradation products such as indole and skatole [2]. In 2010, leading associations of the German agricultural and meat industry as well as the food trade agreed in the "Düsseldorf Statement" committed to abandon this method. A current EU legislation enforces the ban of surgical castration of piglets without anesthesia from 2019 onwards [3]. Consequently different alternatives to surgical castration without numbing are discussed and examined. One of these alternatives is boar fattening. Consequence it is speculated that the percentage of off-flavours might increase. Regardless of selecting an adequate alternative there is an urgent requirement for an analysis procedure. To check whether an alternative has brought the desired success, a dependable quantification of odour components must be guaranteed, allowing a judgement. In this work, a solidphase-microextraction-GC/MS-method (SPME–GC/MS) was implemented, according to [4]. The method includes a methanolic extraction of melted fat, additon of deuterated labeled standards of the analytes, a freezing step for separation of fat and solvent and a subsequent solvent evaporation. The method has been validated by determining precision and recovery data as well the detection limit. The method proofed sensitive enough to detect trace levels below the order thresholds. Analytical control measures have been implemented to ensure reliable and precise analytical results. To support a national monitoring program a report of 289 back fat samples were examined and subsequently evaluated.

[1] EFSA; Welfare aspects of the castration of piglets, Question No EFSA-Q-2003- 091; 2004
[2] Dijksterhuis, G. et al.; An international study on the importance of androstenone and skatole for boar taint; Meat Science; 2000; Vol. 54; pp. 261-269
[3] European Commission; European Declaration on alternatives to surgical castration of pigs; 2010
[4] Fischer, J. et al.; Development of a Candidate Reference Method for the Simultaneous Quantitation of the Boar Taint Compounds Androstenone, 3α-Androstenol, 3β- Androstenol, Skatole, and Indole in Pig Fat by Means of Stable Isotope Dilution Analysis- Headspace Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry

Keywords: *boar taint, androstenone, skatole, spme*

E10
MANUFACTURE AND SENSORY, PHYSICOCHEMICAL PROPERTIES OF A PROBIOTIC YOGURT FLAVORED WITH PUMPKIN SPICE SYRUP

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A probiotic yogurt was prepared using whole cow milk as raw material (milk fat 4%, protein 3.5%), a starter culture of ABT-5, CHR-Hausen, Denmark and skim milk powder to increase solids of milk. The flavour of the probiotic yogurt was obtained by using pumpkin spice syrup at different rates (3; 5 and 6% (*w/w*)). Yogurt samples were analyzed for physical, chemical, microbiological and sensory characteristics. There were significantly differences in the fat, ash, protein, total solids content and titratable acidity for samples due to different added rates of pumpkin spice syrup. As a result of the organoleptic evaluations, probiotic yogurt samples containing 6% pumpkin spice syrup had the highest overall acceptability. Also the same samples had higher flavor scores than of those using smaller rates of flavour additive. A mean score between six and seven indicated that the sample product was “like” accepted. The total aerobic mesophilic bacterial count, coli form count, and yeast and mold counts were determined in yogurt samples at 1; 7; 14 and 21 days interval. Volatile components of pumpkin spice syrup were extracted using a mixture of 1:1 acetone: diethyl ether, dried and concentrated followed by analysis using gas chromatography coupled to mass spectrometry.

Keywords: *probiotic yoghurt, pumpkin spice syrup, flavouring, acceptability*

E11
WHAT'S IN YOUR MORNING DRINK? COMPREHENSIVE CHARACTERISATION OF COFFEE AND TEA EXTRACTS BY GC×GC-TOF MS

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Almost one thousand different compounds have been identified in roast coffee extracts, with chemical composition varying due to a range of factors, such as coffee bean origin and degree of roasting. The overall flavour and aroma results from a complex combination of chemical classes, including terpenes, oxygenates (aldehydes, esters and ketones) and thiophenes, as well as a range of nitrogen-containing compounds (pyrazines, pyridines and thiazoles). Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) is ideal for the analysis of complex samples, such as coffee and tea. The enhanced separation capacity of this technique allows the entire sample characterisation within a single run. In this study, key flavour compounds, which would have been subject to extensive co-elution in a conventional GC–MS system, were quickly and confidently identified using automated search tools. For example, simple scripting functions were applied to allocate the pyrazines, pyridines and thiazoles based on their characteristic fragmentation patterns, allowing fast characterisation of the sample.

Keywords: *GC×GC–TOFMS, tea, coffee, aroma, time-of-flight*

E12
MULTIPLE HEADSPACE-SOLID PHASE MICROEXTRACTION APPLIED TO THE QUANTIFICATION OF VOLATILE COMPOUNDS IN MERLOT WINES

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Aroma is one of the most important factors that determine the character and quality of a wine. Hundreds of chemically different compounds, including alcohols, esters, acids, terpenes and others have been found in wines. This determines the complexity of the matrix, as the volatile compounds have different polarities, volatilities and are found in a wide range of concentrations. An alternative to eliminate the matrix effects in complex samples is the multiple headspace extraction (MHE), which consists in the dynamic gas extraction procedure carried out stepwise. In this method, the area value of each analyte obtained by a complete extraction depends exclusively on the amount of analyte and not on the matrix composition. A Multiple headspace-solid phase microextraction (MHS–SPME) followed by a gas chromatography coupled with a mass spectrometry (GC–MS) was applied for the identification and quantification of volatile compounds present in red wines of the varie ty Merlot. Twenty five esters, two alcohols and five terpenes were analyzed. The MHS was performed through four consecutive extractions, with five minutes interval between each of them. The method's performance was tested by evaluating the detection limit (0.033 to 0.078 μg L^{-1}), quantification limit (0.111 to 0.259 μg L^{-1}), recovery (from 92.3 to 108.5%), accuracy (0.82 to 11.21%) and linearity (0.9905 to 0.9977). The proposed method allowed for the accurate determination of a group of volatile compounds with an important role in the wine's aroma performed without the matrix effect. Furthermore, this method provides valuable information about the relative ease with which these compounds are transferred to the headspace, since smaller β values indicate higher proportion of compound transferred to the headspace. Significant differences in the relative volatility of alcohol in relation to terpenes were observed. Regarding esters, volatility was related to the length of the carbon chain. The compounds found in highest amount in Merlot wines were ethyl acetate, 2-phenylethanol, ethyl octanoate and ethyl isovalerate, with aromas of fruits, roses, floral and apple, respectively.

Keywords: *volatile compounds, multiple headspace-solid phase microextraction, Merlot red wine, GC–MS*

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E13
EFFECT OF SOLVENT ON THE CAROTENOID EXTRACTION FROM GERBERA JAMESONIC BOLUS OF VIETNAM

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Carotenoids are not only the common natural color, but also have potential applications such as the pro-Vitamin A component. This pigment has good application in the food industry. In this study, various carotenoids extraction from Gerbera Jamesonic Bolus was surveyed to achieve the highest extraction, which is grown in Vietnam. Gerbera Jamesonic Bolus was collected from South of Vietnam. The sample was dried at 60–70°C to A_w=7%, then was ground into flour. After collecting, the ground petals were treated by using different solvents (acetone, methanol, ethanol, hexane) with different number of extraction times (1, 2, 3 times) with different ratio of raw material and selected solvent (1:20, 1:30, 1:40, 1:50, 1:60, (*v/v*)) at different temperature (30, 40, 50, 60, 70°C), and different extraction time (30, 60, 90, 120, 150 min.). The extracted solution was filtered by vacuum filter to remove the sediment. To extract carotenoids, vacuum rotary evaporator was used to get the high color content of the received liquid. (Dam Sao Mai, Le Van Tan, 2013) The quantity of carotenoids was determined by the absorption method as described by Delia (2001). This was simple method that measures the absorption of extracted liquid at wavelength λ = 483 nm. The results were then compared with standards carotenoids (via $K_2Cr_2O_7$ solution) diagram. This study results demonstrated that carotenoids extraction is better when using methanol as solvent with raw material and solvent ratio 1:40, extraction time 60 min., 2 times of extraction and temperature 60°C. This extraction condition was able to extract 694.45 ppm of carotenoids from Gerbera Jamesonic Bolus. The carotenoids are the good components for human health and for food color application; therefore, this extraction method could be applicable in food processing for further researches.

Keywords: *Gerbera Jamesonic Bolus, carotenoids, food color, solvent, pigment extraction*

E14
IDENTIFICATION AND QUANTIFICATION OF AROMA-ACTIVE COMPOUNDS OF ORANGE JUICE FROM THE FCOJ PROCESSING STEPS USING HS–SPME–GC–MS

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Brazil is the largest producer and exporter of frozen concentrated orange juice (FCOJ). The FCOJ processing conditions of temperature and time can affect orange juice aroma and flavour, therefore reducing the overall acceptance. The aim of this work was to identify and quantify the aroma-active compounds of orange juice from the FCOJ processing steps using HS–SPME–GC–MS. Orange juice from Pêra-Rio variety, from the extraction (11.5°Brix), finishing (11.8°Brix), 1st stage of evaporator, concentration and blending steps were used. The juice from the 1st stage of evaporator, concentration and blending steps was reconstituted with water up to 11.8°Brix prior the analysis. Headspace solid phase microextraction (HS–SPME) was performed using a manual holder with a 10 mm length 50/30 µm PDMS/CAR/DVB fibre. 10 mL of juice was transferred to a 20 mL vial with a magnetic stirring bar. The fibre was exposed to the sample headspace at 37±1°C for 25 min under s tirring. Ten compounds were identified and quantified by gas chromatography-mass spectrometry. The internal standard calibration (isopentyl acetate) was used in the quantification, with the juice from the extraction step as matrix, because of its representativeness of orange juice aroma. All the compounds showed a decrease in concentration from the extraction and finishing step up to the end of the FCOJ processing, with statistically significant differences (p≤0.05). Ethyl butanoate was higher in the juice from the finishing step (p≤0.05), being strongly reduced in the 1st stage of evaporator step (p≤0.05). β-pinene was higher in the juice from the extraction step, significantly differing (p≤0.05) from the finishing step. β-myrcene showed similar behaviour as β-pinene, with 3.16 µg/mL in the juice from the extraction step and 1.30 µg/mL in the finishing step (p≤0.05). 1-octanol, linalool and α-terpineol were quantified in the juice from all the FCOJ processin g steps. 1-octanol was higher in the finishing step, followed by the extraction step (p≤0.05). Linalool was higher in the juice from the finishing step, differing (p≤0.05) from all the other steps. α-terpineol was higher in the juice from the finishing step (p≤0.05). β-citronellol, perillaldehyde, and decanal were higher in the juice from the finishing step (p≤0.05). Longifolene was higher (p≤0.05) in the juice from the extraction step, followed by the finishing, 1st stage of evaporator and blending steps. Our results showed that in the 1st stage of evaporator step the majority of compounds are lost, affecting the final product aroma quality. Moreover, the oil phase addition in the blending step could not recover area and levels of the volatile compounds.

Keywords: *HS-SPME, aroma, orange juice, volatile compounds*

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E15
ANALYSIS OF AROMA COMPOUNDS IN BEER BY TD–GC–TOF MS WITH SOFT ELECTRON IONISATION

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Beer contains hundreds of organic ingredients, with concentrations spanning many orders of magnitude. Mono- and sesquiterpenes (C10, C15 respectively) are aromatic hydrocarbons found in the essential oils of various plants, and most notably for the brewing industry, in hops. Hops provide much of the characteristic flavouring of the finished beer, so the terpenes content has major impact on the final aroma and flavour. These compounds have very low odour thresholds, making them challenging to detect analytically. The ability to apply quality control to the raw ingredients and the finished product offers desirable cost and time saving to breweries. A rugged, quantitative technique has been developed for the determination of these flavour compounds in beer and is based on sorptive extraction coupled with thermal desorption gas chromatography time-of-flight mass spectrometry (TD–GC–TOFMS). Sorptive extraction is an enrichment technique designed for the extraction of non-polar, semi-volatile constituents from aqueous samples. The approach is simple, quick and economical, like solid-phase micro-extraction (SPME), but sorptive extraction media are physically much larger and can therefore allow higher levels of enrichment for amenable compounds. Coupling this with highly sensitive TOF MS detection and novel soft ionisation technology, ensures that a comprehensive flavour profile can be collected in a single sequence.

Keywords: *time-of-flight mass spectrometry, soft ionisation, thermal desorption, aroma, beer*

E16
THE USE OF HIGH DEFINITION TD–GC–TOF MS FOR CHALLENGING ANALYSES IN THE FOOD INDUSTRY

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Aroma profiles of food contain a wide variety of components at a range of concentrations. Detection and identification of important keynote compounds with a low odour threshold and those responsible for off-odours is a challenging prospect. The use of high definition gas chromatography coupled with time-of-flight mass spectrometry (GC–TOFMS) combined with thermal desorption (TD) offers an ideal solution for routine analysis. Low detection limits and fast acquisition speeds allow trace components, including adulterants and odour taints, to be identified even within the most challenging of matrices. TD brings the added advantage of retaining part of the sample for re-analysis, allowing confident and secure repeat analysis. Additionally, this presentation will explore the use of soft electron ionization by Select-eV technology. Select-eV provides hassle free soft ionisation, with no requirement for source-switching and no inherent loss in sensitivity. The ability to provide enhanced molecular ions whilst retaining structurally-significant fragment ions provides complementary spectra for enhanced confidence in compound identification. An overview of this novel TD–GC–TOFMS system will be presented using specific case studies on aroma profiling of food products.

Keywords: *thermal desorption, time-of-flight MS, aroma, soft ionisation*

FOOD CONTAMINANTS (ENVIRONMENTAL)

(F1 – F52)

F1
THE PREVALENCE OF BACTERIAL CONTAMINATION AND MICROBIAL DIVERSITY USING 16S RRNA GENE SEQUENCING, OF COMMERCIAL EGGS FROM RETAILS MARKET IN SCOTLAND

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In this study, the aim was to describe bacterial diversity of table eggs using both culture and molecular approach. Total viable counts (TVCs) were obtained from shell and content of 88 commercial eggs in Scotland. Eggs from 3 different sources were sampled including organic farm (22 eggs), free range (33 eggs), and caged system (33 eggs). Free range eggs had higher TVCs isolated from eggshell, a mean of 5.5 $\log_{10}$ CFU/eggshell, and 5.2 $\log_{10}$ (caged eggs) CFU/eggshell. Egg content ranged from 3 log (organic egg) to 2.4 log (caged egg) cfu/ml. ANOVA test showed no significant difference between the two variables, TVCs and housing system, for both eggshell, and content respectively (p <0.14, 0.59).
59 bacterial isolates were genotyped by 16SrRNA sequencing. The results obtained indicate large number of eggs inspected was contaminated with *Staphylococcus* bacteria. Among the bacterial strains isolated, *S*taphylococcus *equorum* was the most occurring strain (32%), followed by *Micrococcus luteus* (17%), and rest of the sequences were less than (10%). No evidence was found for presence of *Salmonella*, *Escherichia coli, Campylobacter,* Listeria *monocytogenes*, or *Clostridium perfringens*. The proportion of Gram-positive bacteria was significantly higher than Gram-negative bacteria (p<0.05). It can be concluded that table eggs sold in Edinburgh's groceries were of good quality for human consumption.

Keywords: *egg, bacteria, DNA, PCR, isolation*

F2
QUANTIFICATION OF POLYBROMINATED DIPHENYL ETHERS AND NOVEL BROMINATED FLAME RETARDANTS IN FOOD ITEMS BY GC/ECNI–MS

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Brominated flame retardants (BFRs) are chemicals used in a wide range of commercial and household products in order to reduce their flammability. Because most BFRs are not chemically bonded to the products which they are added to, they can easily leach into the environment. The main exposures of the population to BFRs is via the diet, inhalation of indoor air and ingestion of indoor dust. In particular, the lack of data on the presence of BFRs in food can lead to a wrong estimation of the human intake. The main aim of this project is to follow up the EU Commission Recommendation 2014/118/EU on the monitoring of BFRs in various food items from the Belgian market, to provide data on their presence and levels. The main purpose of the present work was to develop and validate a sensitive analytical method for the quantification of polybrominated diphenyl ethers (PBDEs), novel BFRs (HBB, BTBPE, DBDPE, TBB, TBPH), tribromoanisole (TBA), and Dechlorane plus isomers (syn-DP and anti-DP) at the levels required by the EU regulation in various food items. The method was developed and optimized using three different matrices (lyophilized salmon fillet, chicken breast, and chicken eggs) by modifying the method published by Xu et al. (1). After extraction with acetonitrile:toluene (9:1, *v/v*), the obtained extract was evaporated to dryness, reconstituted in hexane and subjected to Florisil clean-up, and then to an additional acidified silica (5%, w/w) clean-up. The final extract was concentrated to nearly dryness, reconstituted in isooctane:toluene (9:1, *v/v*) and injected into the GC/ECNI system. The analysis of the target analytes was performed with an Agilent 6890 GC, equipped with electronic pressure control and a programmable-temperature vaporizer (PTV) inlet, coupled to an Agilent 5973 MS operated in electron capture negative ionization mode and equipped with a DB-5 capillary column (15 m×0.25 mm×0.10 µm). The reliability of the method was then tested on several food matrices, including different types of fish, meat, eggs, milk, vegetarian food, and vegetable oil (n=58). For all the tested matrices, the recovery rates for all the analytes ranged between 83% ±11 and 104% ±13. The method was finally validated for a variety of food categories (including fish, meat, eggs, milk, and vegetable oil), and the following parameters (linearity, LOQ, trueness, selectivity, repeatability, accuracy, and measurement uncertainty) were evaluated according to the guidelines of ICH and FDA. Based on the obtained results, the developed method was found suitable for the analysis of PBDEs and novel BFRs in different food matrices by GC–MS.

[1] Xu F, García-Bermejo Á, Malarvannan G, Gómara B, Neels H, Covaci A. Multi-contaminant analysis of organophosphate and halogenated flame retardants in food matrices using ultrasonication and vacuum assisted extraction, multi-stage cleanup and gas chromatography-mass spectrometry. J Chromatogr A. 2015, 1401:33-41.

Keywords: *PBDEs, novel BFRs, food items, monitoring program*

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F3
MICROEXTRACTION BY PACKED SORBENT COUPLED WITH GASCHROMATOGRAPHY – MASS SPECTROMETRY: A HYPHENATED TECHNIQUE FOR POLYCYCLIC AROMATIC HYDROCARBONS DETERMINATION IN WATER

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In this work, two different extraction procedures for the analysis of different polycyclic aromatic hydro-carbons (PAHs) in water by microextraction by packed sorbent (MEPS) have been compared in terms of sensitivity, reliability and time of analysis. The first method, called "draw-eject", consists of cycle sequences of aspirations and injections in the same vial; the second one, called "extract-discard", consists of a similar cycle sequence, but in this case the aspired sample is discarded into waste. The relevant partition equilibriums and extraction rates have been calculated by multivariate regression from the data obtained after MEPS gas chromatography–mass spectrometry (MEPS–GC–MS) analysis of 16 PAHs from water samples. Partitioning parameters for a priori prediction of solute sorption equilibrium, recoveries and preconcentration effects in aqueous and solvent systems have been calculated and compared for the two extraction procedures. Fin ally, real samples from sea, agricultural irrigation wells, streams and tap water have been analyzed. Detection (S/N ≥ 3) and quantification (S/N ≥ 10) limits were calculated for the extraction processes. Under the experimental conditions used for the "draw-eject" procedure, these values were in the range 0.5–2 ng L^{-1} and 1.6–6.2 ng L^{-1}, while for the "extract-discard" procedure they ranged from 0.2 to 0.8 ng L^{-1} and from 0.8 to 2.0 ng L^{-1}, respectively.

Keywords: *mIcroextraction by packed sorbent (MEPS), polycyclic aromatics hydrocarbons, water contaminants, mathematical model*

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F4
EVALUATION OF RESULTS OF EU-RL PROFICIENCY TESTS ON DETERMINATION OF PCDD/FS AND PCBS IN FEED

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The EU-RL for Dioxins and PCBs in Feed and Food organizes proficiency tests (PTs) for determination of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), dioxin-like PCBs (DL-PCBs) and indicator PCBs (PCB 28, 52, 101, 138, 153 and 180) in food and feed matrices for National Reference Laboratories (NRLs) of EU member states and official laboratories twice a year. The objective of these PTs is to assess analytical performance of laboratories, the interlaboratory comparability of results from analyses of relevant parameters and, if necessary, the inclusion of other important aspects as the reliability of analytical results for PCDD/Fs and dioxin-like PCBs provided by GC–MS/MS methods. Specific PTs are also open for other official laboratories and commercial laboratories in order to check the comparability of results not only within the EU-RL/NRL/OFL network, but also within official and private laboratories performing official control or self-contro l of feed business operators. Analytical criteria for determination of PCDD/Fs and PCBs are established in Commission Regulation (EC) No 152/2009. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed sets maximum levels and/or action thresholds for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ and sum of six indicator PCBs in various feed materials ranging from compound feed to different feed additives. Contaminated feed samples can cover a wide range of different congener patterns for PCDD/Fs and PCBs depending on the source of contamination or the general background levels. For PCDD/Fs also non-2,3,7,8-chlorinated congeners can be present in considerable concentrations. Feed samples used for EU-RL PTs were either naturally contaminated or spiked with relevant PCDD/F and/or PCB congeners in order to get PT samples contaminated in the range of established maximum levels and/or action thresholds. Between 2013 and 2015 three EU-RL PTs covering the following four feed matrices were performed: feed fat, sepiolite, compound feed for fish and sugar beet pulp. Between 83 and 120 participants reported results for at least one of the requested parameters. The statistical evaluation of results is performed according to ISO 13528:2005 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (IUPAC Technical Report, Pure Appl. Chem, Vol. 78, No. 1, pp.145–196, 2006). The evaluation of the results of the proficiency tests showed, that either levels of analytes of interest in the samples, specific properties of the sample matrix or specific interferences can have a profound influence on the results. In case of the sugar beet pulp sample, which was contaminated by an open drying process, interferences with non-2,3,7,8-subsituted PCDD/Fs have to be considered. This resulted in considerable variations of results for some individual congeners and WHO-PCDD/F-TEQ.

Keywords: *proficiency test, dioxins, PCBs, feed*

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F5
QUANTIFICATION OF BROMINATED FLAME RETARDANTS IN FOOD BY UPLC-MS/MS: CHALLENGES, ANALYTICAL METHOD DEVELOPMENT AND VALIDATION IN FOOD

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The study was undertaken in order to respond to the Commission Recommendation 2014/118/EU on the monitoring of brominated flame retardants (BFRs) in food in Europe (1). BFRs are anthropogenic chemicals that are added to a wide variety of consumer products in order to improve their fire resistance. BFRs may slowly leak from the products into the environment. Due to their persistence and potential to bioaccumulation in the food chain, BFRs may cause adverse effects in humans and animals. There is a lack of information on the occurrence data of BFRs in food which has hampered accurate completion of intake assessment. The main objective of the work was to provide a sensitive analytical method for quantification of brominated phenols (BrPh) and hexabromocyclododecanes (HBCDs) in foodstuffs. Measurements of BFRs were performed using ultra-high performance – tandem mass spectrometry (UPLC–MS/MS) technique on ACQUITY UPLC system (Waters) coupled to Xevo-TQ-S mass spectrometer (Waters). The MS was operated in electrospray ionization mode in negative polarity. In addition to its selectivity and superior sensitivity, the LC–MS technique was chosen over gas chromatography-mass spectrometry (GC–MS) because of applicability for compounds susceptible to thermal decomposition (derivative of tetrabromobisphenol A) and isomeric interconversion (HBCD isomers). The optimisation of sample preparation procedure was performed using lyophilized salmon as matrix. Spiking experiments demonstrated good results applying acetonitrile as extraction solvent. The extract was defatted using consequent addition of hexane. To enhance phase separation between hexane and acetonitrile, addition of water was required. The organic phase containing hexane was brought onto a multi-layer silica column (including layers of acidified silica) for lipid elimination. For solvent exchange, the aqueous phase was cleanedup by solid-phase extraction using Oasis® HLB cartridges. After the clean-up, the extracts were evaporated, reconstituted in acetonitrile/water (50/50) and combined before injection into the UPLC–MS/MS system. In this work, challenges encountered in the mass spectrometric detection of BFRs, as well as considerations in extraction and quantification of these compounds will be discussed. Validation of the developed UPLC–MS/MS method will be presented for a variety of food groups, including fish, meat, eggs, milk and vegetable oils.

[1] Commission Recommendation of 3 March 2014 on the monitoring of brominated flame retardants in food (2014). Official Journal of the European Union, L65: 39-40.

Keywords: *brominated flame retardants, food, monitoring, UPLC–MS/MS*

Acknowledgement: *Financial support from Federal Public Service (FPS) Health, Food Chain Safety and Environment*

F6
IDENTIFICATION AND DETERMINATION OF TOXIC SUBSTANCES (PESTICIDES AND MYCOTOXINS) AND THEIR TRANSFORMATION PRODUCTS IN NUTRACEUTICALS BY UHPLC–ORBITRAP–MS

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Nutraceuticals can be considered an alternative to pharmaceutical products by society, and they have been acquiring importance in the last decade. Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered foods, herbal products, and processed foods such as cereals, soups and beverages. Keeping in mind that a nutraceutical product is a concentrated form of a food or plant, it can be contaminated from the raw material with toxic substances, such as pesticides or mycotoxins. In the European Union, there exist legislation involving maximum residue limits for pesticides [1] and mycotoxins [2] in food and feed, although they only consider their presence in the raw material and not in the nutraceutical presentation (capsules, tablets, extracts, etc.). Another problem is that these contaminants can be degraded to other substances, which in some cases could be more toxic than the original ones. These are considered transformation products (TPs). Most of these TPs are unknown and few studies have been performed to analyze them. Therefore, analytical methodologies that ensure food safety in this kind of commodities are needed, monitoring toxic substances as well as their respective TPs. In this work, different nutraceuticals, such as green tea, royal jelly, soy isoflavones and ginkgo biloba, have been studied looking for more than 350 toxic substances (including pesticides and mycotoxins) using ultra high pressure liquid chromatography coupled to high resolution mass spectrometry (UHPLC–Orbitrap-MS). A “dilute and shoot” extraction procedure was applied for all the matrices studied, followed by a clean-up step using different sorbents, such as primary secondary amine (PSA), graphitized carbon black (GCB), C18, Florisil and zirconium oxide (Z-Sep+). The methodologies were validated, obtaining recoveries between 70 and 120% and precision values below 20%. The limits of detection and quantification were below 10 and 20 µg kg-1, respectively. After validation, the methods were applied to different commercial nutraceutical products (36 in total), finding 28 pesticides and 4 mycotoxins. TPs were defined for each toxic substance found in the real samples, building a list of 31 TPs. From this list, 5 TPs were putatively identified by Orbitrap-MS in the commercial nutraceutical products analyzed, performing retrospective analysis.

[1] REGULATION (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.
[2] REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants and foodstuffs.

Keywords: *nutraceuticals, pesticides, mycotoxins, transformation products, high resolution mass spectrometry*

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F7
ASSESSMENT OF THE BIOACCESSIBILITY OF PCBS IN MEAT AFTER DIGESTION

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Various toxic micro-pollutants like the polychlorinated biphenyls (PCBs) are frequently found in food especially in animal-based food such as meat, thus representing a public health risk. The risk assessment of these toxic contaminants is crucial for both producers and consumers. Today, this risk assessment is based on the contaminant content in food. Recent researches suggested that the digestion could significantly modulate the fraction of contaminants actually assimilated by the consumer's body, ie the bioaccessible fraction. Therefore, in order to better evaluate the bioaccessibility of contaminant, it is necessary to set up in vitro digestion processes for simulating the principal steps of the digestion. The aim of this study is to develop a protocol to evaluate the bioaccessibility of PCBs in meat after the digestion. Firstly, a standardized static in vitro digestion method recently proposed by a scientific community was adapted and assessed. This me thod included the salivary, gastric and intestinal steps. Secondly, an extraction method of PCBs based on accelerated solvent extraction (ASE), centrifugal evaporation and gel permeation chromatography (GPC), was developed to optimize (i) the recovery rates of PCBs after digestion and (ii) their analysis by GC×GC–TOF/MS. Thirdly, based on the results obtained so far, the bioaccessibility of 18 relevant PCB congeners in beef was evaluated after in vitro digestion. Finally, this protocol was expanded to study several factors which could have impact on the bioaccessibility of contaminants, such as the fat content of meat, the mode of cooking and the physiological states of consumer (adult vs infant).

Keywords: *food safety, bioaccessibility, PCB, In vitro digestion, Mmeat*

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F8
PAHS IN MAINSTREAM SMOKE – CHALLENGES FOR ANALYTICAL METHODS?

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Organisations in different regions of the world, including parties to the World Health Organisation Framework Convention on Tobacco Control (WHO FCTC) and the US Food and Drug Administration (FDA) have specified toxicants in mainstream cigarette smoke and tobacco that are of regulatory interest, including Polycyclic Aromatic Hydrocarbons (PAHs). Currently, benzo[a]pyrene has been prioritised by the Conference of Parties to the FCTC and the US FDA list of 18 Harmful and Potentially Harmful Constituents (HPHCs) for regulatory data submission. However, the full FDA HPHCs list of 93+ constituents including 16 PAH substances may have to be reported in due course. The measurement of PAHs in mainstream smoke is technically challenging because of their relatively low abundance (parts per billion), numbers of isomers and the consequent need for high chromatographic and detection selectivity. Their determination requires not only thorough optimisation of the extraction and clean-up strategy but also the application of appropriate instrumentation to give sufficient selectivity and sensitivity. The presented study discusses some challenges associated with optimisation of targeted analytical methods for determination of PAH substances in mainstream cigarette smoke. As an example, an analytical approach comprising stable isotope dilution MS, extraction using Accelerated Solvent Extraction (ASE), a dual Solid Phase Extraction (SPE) clean-up and a comparison of three different GC/MS systems (high resolution magnetic sector (GC–HRMS), triple quadrupole (GC–MS/MS) and single quadrupole (GC–LRMS) mass analysers) is presented. Practical aspects of method development as well as advantages and limitations of all three systems are discussed. GC–HRMS was demonstrated to be the most sensitive technique for quantitative analysis of PAHs present at sometimes (ultra)low concentration levels in mainstream cigarette smoke.

Keywords: *polycyclic aromatic hydrocarbons, mainstream smoke, accelerated solvent extraction, high resolution magnetic sector, triple and single quadrupole mass analysers*

Acknowledgement: *This work was funded by British American Tobacco. The authors would like to thank Marchwood Scientific Services for conducting the study.*

F9
SIMULTANEOUS DETERMINATION OF PCDD/FS, PCBS, PBDES IN EGGS USING AUTOMATIC PREPARATION SYSTEM

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Food inspection for the content of toxic PCDD/Fs, PCBs and PBDEs has a significant impact on consumer safety. Analytical criteria required for dioxins and PCBs analysis in food are contained with the EU and US EPA legislation. Methods of chemical analysis of these commonly occurring pollutants are time consuming and therefore simultaneous detection and determination of 35 dioxin and PCB congeners together with 12 PBDEs congeners (BDE-28, 30, 47, 49, 77, 99, 100, 138, 153, 153, 183 and 209) was the aim of our study. As a matrix chicken eggs were used. Egg samples were selected due to special attention paid recently to free range eggs because their frequent pollution with dioxins. To meet objective of the study pressurized liquid extraction (PLE) and automatic clean-up procedure were utilized. Eggs extraction were carried out at the temperature of 120 OC and pressure of 1600 psi for two cycles with solvent mixture methanol/toluene 70:30 v/v. Extract purification was done by four chromatography columns: silica gel modified with acid, base silica, activated alumina, and activated carbon. Two fractions were obtained: dioxins, furans and non-ortho PCBs were present in the first fraction, and mono-ortho PCBs and PBDEs ware in the second one. Both fractions were analyzed by isotope dilution method (IDMS) with combined technique HRGC–HRMS. We didn't find any statistical difference between results obtained by automated and routine method used on regular basis in our laboratory (Mann-Withney test p≤ 0.01). Sample analysis time was reduced six-fold from about 80 to 15 working hours. The parameters of developed analytical methods were as follows: quantification limits (LOQ) for PCDD/Fs and PCDD/F/DL-PCBs were 0.16 and 0.19 pg WHO-TEQ/fat respectively and 0.19 ng/g for NDL-PCBs. Method recoveries were in the range 30 to 119%, trueness from 13 to 2%, and the within-laboratory reproducibility (RSDR) was less than 7%. LOQ of PBDEs was between 0.01and 0.06 ng/g fat for tri to hepta brominated congeners and 0.91 ng/g fat for BDE-209. Method recoveries for the tested flame retardants (PBDEs) were 78-95% and within-laboratory reproducibility (RSDR) was below 9%. BDE-30 was an exception for which the level of method recovery was 63 % and within-laboratory reproducibility (RSDR) 25%. The developed method meets the criteria required for determination of PCDD/Fs, PCBs, PBDEs in food and let to significant reduction of analysis time. Successful participation in proficiency test organized by European Reference Laboratory for dioxins and PCBs, confirmed that the developed method fits for the intended use. (EN ISO/IEC 17025:2005).

Keywords: *method, dioxins, PCB, PBDE, HRGC-HRMS*

F10
SIMPLIFIED SOLID PHASE EXTRACTION CLEANUP AND ANALYSIS OF POLYAROMATIC HYDROCARBONS IN BUTTER USING GC/MS

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Polyaromatic hydrocarbons (PAHs) are ubiquitous in the environment, resulting from both natural and manmade sources of combustion. Farm animals, such as cows, can become exposed to them through both consumption of contaminated feed and water, and inhalation of contaminated atmosphere. Consequently, these compounds can be present in dairy products produced from cow's milk such as cheese and butter. Analysis of butter can pose a special challenge due to its high fat content combined with the lipophilic nature of PAHs.
In this application, extraction of 28 different PAHs from butter was achieved using a dual-layer solid phase extraction (SPE) cartridge containing Florisil and a mixture of two different surface-modified silicas. The subsequent extract was then subjected to cleanup by passing it through a small silica gel SPE cartridge. This produced a final sample with background low enough to allow for analysis on a single quadrupole GC/MS system. Using the described method, recoveries from spiked butter were in the range of 80–120% for a majority of the targeted PAHs, with RSD values of <10% for replicate samples.

Keywords: *PAH, food contamination, fatty samples, sample preparation, SPE*

F11
INVESTIGATION OF HEAVY METAL CONTENTS BY MILLING DEGREES OF RICE

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Recently, various rice by milling degree is sold for health and taste. To provide safe food to consumers, it is need to know the change of heavy metal contents according to milling degree of rice with exceeding the MRL of Cd and Pb. Rice were milled by five degrees (0.0, 2.45, 8.02, 10.48, 15.09%). Milled rice were digested by microwave method, and analyzed heavy metal contents using ICP-OES. Recovery ratio of 4 heavy metals such as Cd, Pb, Cu and Zn were ranged for 79.7–98.9%, and also limits of detection(LOD) and Limits of quantitation(LOQ) were fulfilled the normal analytical standards. Cadmium (Cd) and Lead (Pb) were detected 0.416–0.433 mg/kg and 0.183–0.26 mg/kg, respectively. From these results, it was known that almost of the samples exceeded the MRLs showed still exceeded the MRLs after milling. Also it was detected 3.639–3.882 mg/kg in Copper (Cu), and 16.868–19.801 mg/kg in Zinc (Zn). Generally speaking, from the results of the investigation the contents of heavy metals were decreased related to the milling degree. From the results of this investigation the contents of heavy metals were decreased related to the milling degree. Rice with high level of contamination by even a kind of heavy metal is possible to crossed contamination of other heavy metals. This rice could be not safe after milling regarded as the MRLs. And it is necessary to manage the risk assessment for heavy metals using more rice samples.

Keywords: *cadmium, ICP–OES, lead, milling degree, rice*

F12
THE LEVELS OF HYGIENE INDICATOR MICROORGANISMS AND FOOD POISONING BACTERIA IN MARINE FOODS

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This study was carried out to update the current specifications of Korea Food Code for hygiene indicator microorganisms and food poisoning bacteria in foods: seafood such as fresh or frozen sasimi and dried laver. A total of 400 samples were collected from a number of grocery stores and markets. Aerobic viable bacteria, coliforms and E. coli as hygiene indicator microorganisms were tested with the method of 'Dry sheet medium culture plate' and 'MPN' specified in the Korea Food Code. Staphylococcus aureus, Vibrio parahaemolyticus, Listeria monocytogenes, Salmonella spp as food poisoning bacteria were analyzed with methods specified in the Korea Food Code. Each sample was tested five times according to ICMSF (International Commission on Microbiological Specifications for Foods) standard. The number of aerobic viable bacteria and coliforms was appeared much higher in dried laver than fresh or frozen Sasimi.

Keywords: *food, standard, hygiene, microorganism, ICMFS*

F13
COMPARATIVE STUDY ON THE ANALYSIS OF PCDD AND PCDF IN FOOD AND ANIMAL FEED USING GC–MS/MS AND GC–HRMS TECHNIQUE

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Polychlorinated Dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) showing adverse health effects to human being. A significant part of the intake can be assigned to a contaminated food chain. The current methods to determine the amount of dioxins and dioxin like substances are described in European legislations. The standard technique to confirm PCDD, PCDF and dl-PCB levels in feed and food is gas chromatography (GC) coupled to high-resolution mass spectrometry (HRMS). In a revision of this directives published in 2014 the European commission allowed to use the more cost efficient triple quad technology (GC–MS/MS) also to confirm the concentration of these compounds in food and feed. Therefore, the objective of this work was to investigate the data quality achievable with GC–MS/MS and to show comparability with GC–HRMS measurements. More than 50 real world samples and corresponding standards were prepared using isotopic dilution technique. After the clean-up process two recovery standards were added to check for recovery criteria given in European regulation. The prepared samples were analyzed with GC–MS/MS as well as with GC–HRMS instrumentation to determine the WHO established TEQ-value. The chromatographic column was a 5% phenyl with 60 m, 0.25 mm, 0.1 µm film MS column. Injection volume was 2 µl into a split/splitless injector in splitless mode maintained at 280°C. GC oven temperature started at 130°C for 1 min, 20°C/min to 190°C, 8 min, 2°C/min to 220°C, 3 min, 6°C/min to 244°C. Each compound was measured with 2 MSMS transitions using unit/unit resolution for the tandem mass spectrometer. The calibration ranges used were 0.1–10 pg/µl for Tetra and Penta, 0.2–20 pg/µl for Hexa and Hepta, 0.5 50 pg/µl for OCDD and OCDF with R^2 > 0.999. Eight replicates were done on the lowest standard giving an RSD% below 3%. Different types of real world sample matrices like fish oil, porc fat, eggs, milk, and animal feed were investigated. All samples showed a very good correlation between GC–HRMS and GC–MS/MS results. A maximum deviation in TEQ values between GC-MSMS and GC–HRMS below 10% was observed. It is noteworthy that this high level of comparability was even reached at levels below 0.5 ng/kg. The data shown in this publication indicate that the use of GC–MS/MS technology for dioxin screening and concentration confirmation is a robust, reliable, and cost efficient alternative to the use of GC–HRMS technology.

Keywords: *dioxins, food and feed, GC–MS/MS, GC–HRMS, POP*

F14
WHAT DOES THE EVER LOWER CHEMICAL RESIDUE CONCENTRATION CHALLENGE ENTAILS FOR ANALYTICAL CHEMISTS IN TERMS OF CONTAMINATION HANDLING AND DATA GENERATION?

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Since the mid-1990s, with the inclusion of "dioxin-like" polychlorobiphenyl (DL-PCB) (DL-PCB) in the risk management measures associated to dioxins (PCDD/F) in human food and animal feed, the number of laboratories involved in ultra-trace analysis (sub $ng.kg^{-1}$ level) has been significantly increased. A certain number of dedicated precautions are taken by official laboratories in accordance with the EU/USA regulation. Indeed, quantification is based on isotopic dilution in agreement with EPA 1613. Quality controls including method blanks are used to prevent any cross-contamination possibly occurring during the analytical process. However, the ultra-sensitivity reached by the current technologies regularly reveals contamination for some congeners. Their origins are multiple and have to be methodological explained to be well managed [1]. Same observations are reported for most of the contaminant classes e.g. brominated flame retardants (BFRs) [2,3], phthalates [4,5] or nonylphenol [6]; analytical equipments as well as consumables (phases, solvents...) found and used in laboratories have been pointed out as a major sources of samples contamination. Ultra-pure water used for sample extraction and purification has been demonstrated to be a critical source of contamination for e.g. perfluoroalkyl compounds or bisphenol A [7]. The production of reliable analytical data for ubiquitous contaminants necessitates defining a systematic strategy to identify, to reduce and to control potential sources. Moreover, clear rules must be established to take into account potential residual contribution in the produced data. For instance, the strategy consisting into the subtraction of the contamination level observed in the method blank should be clearly considered, validated and communicated. This attitude is applicable both to risk management (compliance declaration) and risk assessment (total diet studies) operations. We propose to share on this poster 1) our reflexion considering possible strategies for contamination handling (source characterization, contamination limitation and monitoring), and 2) the way to express the data to the authorities or sanitary agencies when the hazard is susceptible to be cross-contaminated by indoor laboratory atmosphere or materials.

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Keywords: *ultra trace level, reliable analytical data, blank contamination, dioxins, BFRs*

F15
ANALYTICAL STRATEGIES AIMING AT COVERING PBDES, PBBS, HBCDDS, TBBPA AND NEW BROMINATED FLAME RETARDANTS IN FOOD

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Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs) have been widely used for years. They are now present in the environment and a large range of food and feeding stuffs and are becoming a concern for human health. The EU ban of certain technical mixtures such as pentaBDE or octaBDE led manufacturers to turn towards new compounds in replacement for PBDEs and HBCDDs which were splitted by EFSA [EFSA Journal 2012;10 (10):2908] into two categories: emerging (chemicals which are applied as flame retardants and that have been identified in any environmental compartment, in wildlife, in food or in humans) and novel (defined as chemicals applied as flame retardants, with confirmed presence in materials and/or goods in concentrations above 0.1 %, but not identified in environmental samples, wildlife, food or humans). The lack of information regarding these compounds is often linked to the non availabi lity of dedicated analytical method to establish their occurrence in the environment, biota or human. The aim of this work was to develop a unique analytical methodology permitting the simultaneous characterization of PBDEs, PBBs, HBCDDs, TBBPA and emerging/novel e/n-BFRs. In addition to 8 PBDEs (BDE #28, 47, 99, 100, 153, 154, 209), 3 PBBs (52/101/153), 3 HBCDs (α/β/γ) and TBBPA, 16 e/n-BFRs were analyzed (pTBX, TBCT, PBBz, PBT, PBEB, HBBz, OBIND, T23BPIC, BRPs, TCBPA, EHTBB, BEHTEBP, BTBPE, DBDPE, TBBPA-bME and TBBPA-bDiBPrE). The method relies on a first lipid extraction followed by a multi-step purification of the extract on acidic silica gel, Florisil® and carbon columns and a liquid/liquid partitioning. Eventually, three different fractions were injected using GC–EI(+)–HRMS detection (JMS800D, Jeol), LC–ESI(-)–MS/MS (6410, Agilent Technologies), LC-ESI(-)-HRMS (1260, Agilent Technologies/Thermo Exactive) or GC-APCI(+)-MS/MS systems (APGC–Xevo TQS, Waters). The method wa s applied to 200 food samples (meat, liver, milk…) collected in the 2014 French monitoring plan. All targeted PBDEs except BDE#183 were found in these samples. BB 153, α-HBCD and γ-HBCDD were always detected but very often below pg/g ww. TBBPA was mainly identified in seafood. PBT, PBBz, HBBz, BTBPE and BEHTEBP were present in most of the food samples. EHTBB were detected in almost 50% of the food items. TBCT, PBEB, pTBX, DBDPE, OBIND, T23BPIC, TBBPA-bDidBrPE, TBBPA-bME, TeBRPs and PeBRPs have been never found above the limit of quantification.

Keywords: *brominated flame retardants, EFSA Journal 2012; 10 (10):2908, APGC, chemical food safety, risk assessment*

Acknowledgement: *The analysis of French foods was funded by the French Ministry of Agriculture, DGAl*

F16
GAS CHROMATOGRAPHY – ATMOSPHERIC PRESSURE CHEMICAL IONIZATION - TANDEM MASS SPECTROMETRY FOR EMERGING/NOVEL BROMINATED FLAME RETARDANTS MONITORING IN FOOD

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Since 2006, EFSA – the European Food Safety Authority– has published successive recommendations encouraging member states to include brominated flame retardants (BFR) in their national monitoring plans so that the exposure of European consumers is more accurately known. Based on the opinion of EFSA [EFSA Journal 2012;10(10):2908. doi:10.2903/j.efsa.2012.2908], the EU Commission recommended (2014/118/EU) the monitoring of traces of brominated flame retardants in food items including 17 emerging and 12 novels BFR. Their various physicochemical properties (e.g. LogP, MW...) make a multi-residue approach quite difficult if not unachievable: adapted extraction techniques (e.g. volatile versus high boiling point's analytes), multiple purification fractions, different chromatography (e.g. GC vs LC) and various mass analyzers (e.g. MS/MS vs HRMS). The work presented in this poster assessed GC–(APCI)–MS/MS capability to cover the largest range of novel/emerging BFR in the same analytical run. Gas chromatography variables [liner, injection temperature, stationary phases (nature, film thickness, internal diameter, length...), column dimensions, column's end positioning in the source...] were optimised. Eventually, an ultra-inert double gooseneck liner was positioned ahead of a 15 m x 0.25 mm capillary column filled with a 0.1 µm film thickness (Rtx®-1614 from Restek). A de-activated post-column was set up on an Agilent 7890A GC. The APCI source was used in the positive ion mode. Dry conditions were preferred to promote cation radical formation. A 2 µA current Corona was used for better sensitivity and signal stability (ion plasma). Two transitions per compounds (n=30) were monitored (SRM acquisition, precursor ions from *m/z* 172 to 987). The optimized conditions developed on the GC–(APCI)–MS/MS allowed the efficient characterization of a large set of emerging/novel RFB in food as attested by the realization of the 2014's Frenc h monitoring plan. Close attention has to be taken on the cleanliness of the system (chromatography and ion source especially) to minimize risk of rapid sensitivity decreasing.

Keywords: *emerging, exposure, risk assessment, food safety*

F17
LC–MS/MS DETECTION OF THE PESTICIDE 1080 (SODIUM FLUOROACETATE) IN MILK AND INFANT FORMULA

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Recently (November 2014), threats in the form of letters were sent to farming and dairy industry leaders in New Zealand. The letters were accompanied by small packages of milk powder that were shown to contain a concentrated form of the pesticide 1080 (sodium fluoroacetate). The sender demanded that the New Zealand government stop using 1080 for pest control. Sodium fluoroacetate is used to protect New Zealand's native flora and fauna against introduced pests like possums and ferrets. Opponents, however, argue that it also kills native animals and contaminates the environment. Such criminal threats are a potential danger and weaken consumers' trust in the food supply chain. Accurate and reliable analytical methods are needed to monitor food ingredients and final products to ensure food safety in light of this threat. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) is an ideal analytical technique to detect polar analytes in complex food samples. Here we present first results of method development to detect sodium fluoroacetate in milk and infant formula. The sample preparation protocol consists of a simple acetonitrile extraction and defatting using hexane. LC separation was achieved using a HILIC column in normal phase mode. The SCIEX QTRAP® 6500+ system was operated in MRM mode. In MRM mode the transition of a molecular ion into a characteristic fragment ion is monitored. The monitoring of more than a single fragment ion allows not only quantitation but also identification based on the ratio between quantifier and qualifier transitions. Initial studies show that sodium fluoroacetate can be detected at concentrations below 1 ng/mL (below 10 ng/mL in matrix), with good linearity, accuracy, and repeatability.

Keywords: *pesticide 1080, mono fluoroacetate, LC-MS/MS, milk, infant formula*

F18
NON-TARGET AND UNKNOWN SCREENING OF FOOD SAMPLES USING HIGH RESOLUTION LC–MS/MS

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LC-MS/MS is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues and contaminants. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers limits the number of compound to quantify and identify. In addition there is an increasing demand for retrospective non-target (unknown) data analysis to identify unexpected food residues and contaminants. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC–MS/MS run. Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were subsequently analyzed by LC–MS/MS using a SCIEX QTOF system operated in high resolution accurate mass MS and MS/MS mode. Non-Target compounds were identified based on non-target peak finding and sample control comparison to separate chemicals of interest from matrix components. Accurate mass MS and MS/MS information was used to empirically calculate molecular formulae. Found molecular formulae war searched against ChemSpider to find matching structures. Structures were automatically compared against the MS/MS spectrum and theoretically fragmented to tentatively identify the detected compound. Data processing was performed in MasterView software was allows quick processing and intuitive data review.

Keywords: *pesticides, LC–MS/MS, high resolution, TOF, unknown identification*

F19
HEAVY METALS IN PLASTIC, RECYCLING AND ENVIRONMENTAL ASPECTS

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Plastic has become an integral part of our daily life and it's use is increasing. In 2013 the worldwide production has reached an all- time high of about 300 million tons (MT). Single use-packaging account for almost 40%, of the total production. In Europe the produced volume is about 57 MT (demand 46 MT). In 2012, 25.2 million tons of post-consumer plastics ended up in the waste upstream. Of this 26% was recycled while 38% still landed on the landfill [1]. In the past the Life Cycle Assessment (LCA) was linear, after usage it became waste and ended meanly as landfill. Under consumer and political pressure the EU presented a green paper in 2013[2] indicating that it has to become a circular economy. In these economical tough times recycling could create about 160.000 jobs if the recycling rate goes up to 70% in 2020. But is recycling of this single use material easy? Modern packaging materials consist of many layers (up to 7 or more). Each layer has special properties but by physical recycling these properties are lost. Recyclates are becoming a complex mixture. Another complex problem is presented in plastics in durable applications, like cars, electronics, crates and so on. These also have to be recycled but during their functional life new regulations have been introduced. For instance RoHS (2011/65) restricts the maximum concentration of toxic heavy metals in electronic applications (e.g, in end- or consumer products the toxic metal cadmium must not exceed 100 ppm). In the EU several regulations have been developed over the past decades, for instance REACh. The raw materials of the durable recyclates could be contaminated with the inheritance of the past, toxic metals are diluted by physical recycling. Is chemical recycling the future? Nowadays plastic is found littering the environment in large quantities, especially in our seas and oceans. The ingestions of plastic by seabirds have been monitored in stomachs of beached fulmars for more than 30 years. [3]. A reduction of industrial pellets has been observed but in consumer plastic debris no data exists about the toxic load of plastic ingested by wildlife. First investigations prove that heavy metals are present in the plastic fragments.

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[2] EU Commision Greenpaper; On a European Strategy on Plastic Waste in the Environment http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2013:0123:FIN:EN:PDF
[3] Van Franeker, J.A. & Law, K.L. 2015. Seabirds, gyres and global trends in plastic pollution. Environmental Pollution 203: 89-96 (open access). http://dx.doi.org/10.1016/j.envpol.2015.02.034

Keywords: *ICP-OES Spectrometer, ICPESolution s/w, low energy consuption*

F20
MULTI-ELEMENT ANALYSIS OF RELEVANT ELEMENTS AND MACROMOLECULAR CONTAMINANTS IN BLACK POLYMERIC FOOD-CONTACT MATERIAL AND ITS ORIGIN

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Due to globalization, food is no longer a local product, but may be transported over thousands of kilometers from its source to where it is consumed. For this reason, the food needs cooling, sophisticated packaging and preservation to be robust during long transport. Unfortunately, the additives for preservation as well as the packaging material are sources of contamination of food. Strict and steady control from the origin of the food to the final product is needed to protect consumers from undesired contaminations while guaranteeing a high level of quality. From the very beginning, Shimadzu has been involved in the development of analytical methods related to European regulations and following guidelines focusing on consumer protection on a global scale. The common goal is to avoid contamination of air, water, soil and food in order to protect the health and safety of the population. This is achieved by controlling limits of maximum allowable concentrations of hazardous substances. Recent examples are the European drinking water regulation, the European food safety regulations, the recent food and packaging directive and the European wine regulation. Black polymeric food-contact articles (FCA) sold on the European market were measured for their bromine content followed by the identification of presented brominated flame retardants (BFRs) by use of X-ray fluorescence spectrometry (EDX-7000) and thermal desorption gas chromatography coupled with mass spectrometry (thermal desorption GC–MS).In order to confirm the possibility that recycled fractions from the Waste Electrical and Electronic Equipment (WEEE) stream were entering the European market in the form of black polymeric articles, elemental analysis was performed by use of inductively coupled plasma optical emission spectroscopy (ICPE-9800 Series) for the detection of WEEE relevant elements. In most of the BFR positive samples typical elements used in electronic equipment (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sb and Zn) were presented either at trace level or at elevated concentrations.

Keywords: *black polymeric food-contact articles, ICP-OES spectrometry, heavy metals, food contaminants*

F21
STUDY OF FURAN FORMATION DURING COFFEE BREWING

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Furan (C_4H_4O) is a small cyclic ether, classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to human (group 2B) [1,2]. It has been found in many foodstuffs processed by heat treatments [3], where it is formed through multiple pathways, such as Maillard reaction, carbohydrates degradation or lipid oxidation [4,5]. A very popular beverage that is also known as the most contaminated foodstuff by furan is coffee. The high contamination level is known to be related to the bean roasting process occurring at high temperature in anaerobic conditions. Macrae and coworkers in 1985, showed that a small amount of furan precursors remain in coffee even after the roasting and grounding process. The aim of this work is to study the possibility of furan formation in coffee beverages and related cross-products from the remaining traces of precursors within the brewing process conditions. To achieve our objectives, we measured the amount of furan in coffees (in the beverage as well as in the coffee grounds) brewed in air-tightened glass container to prevent furan loss in function of the brewing temperature (in a range between 30 and 90°C) and the coffee brand. For this work we developed a new analytical strategy to accommodate with the very high volatility of the furan. Results obtained by Headspace SPME-Gas Chromatography/Mass Spectrometry and isotope dilution method show that when brewing water heated between 30 and 60°C is used, most type of coffee have the same trends for the evolution of the furan released amount in function of the brewing temperature. This level is staging between 60 and 80°C and finally this trend increases again over 80°C. The only exception we observed is the instant coffee that displays the same released level of furan independently of the preparation extraction temperature. These observations can be explained by one of two hypotheses:
- Furan formation on/in the surface of coffee grounds linked to the temperature increase of brewing water, and furan precursors presence activated when extraction temperature increases.
- Or an improved extraction of furan from the coffee powder.

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Keywords: *furan, coffee, formation, carcinogen, HS-SPME-GC/MS*

F22
QUANTIFICATION OF UV-FILTERS IN SEAFOOD AND MACROALGAE

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UV filters, including both inorganic and organic sunscreen agents, can be found mainly in cosmetics and other personal care products, and also in food packaging, plastics, textiles, and vehicle maintenance products to prevent photodegradation of polymers and pigments. These chemicals can be released to the environment directly via wash-off from the skin or industrial discharges, or indirectly via waste water domestic discharges or releasing by sewage treatment plants; they have been already detected in surface water, sediment and biota [1,2]. The continuous inputs into the environment may lead to toxic effect in human and wildlife, mainly because most of these compounds can act as potential endocrine disruptors. The work presented here deals with the development of a simple and reliable gas chromatography-mass spectrometry (GC–MS) method that enables the simultaneous measurement of 12 UV-filters pertaining to 8 different chemical classes at trace level (ng/L) in seafood and macroalgae. Sample preparation involves liquid–liquid partitioning with acetonitrile in presence of anhydrous MgSO4 and NaCl (QuEChERS) followed by dispersive liquid–liquid microextraction (DLLME) using trichloroethylene as extractive solvent and the extract obtained by QuEChERS as dispersive solvent. Quantification was achieved by GC-MS in selective ion monitoring mode, using deuterated benzophenone as internal standard. For validation purposes, recoveries studies were carried out at two concentration levels (100 and 500 ng/g), yielding recovery rates in the range 70–120% for 75% of the analytes. A good linearity and precision, with relative standard deviations generally below 20% were obtained for all twel ve UV-filters. The lowest method limit detection assigned value obtained was 2 ng/g. The validated method was further applied in the analysis of both seafood and macroalgae collected in four different European polluted areas-hotspots.

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Keywords: *UV-filters, seafood, gas chromatography-mass spectrometry, QuEChERS, DLLME*

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F23
NOVEL LIQUID CHROMATOGRAPHY-MASS TANDEM METHOD FOR QUANTIFICATION OF TETRABOMOBISPHENOL A IN FISH AND BIVALVES

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Tetrabromobisphenol A (TBBPA) is one of the most commonly used flame retardants in the world, namely in the manufacture of epoxy plastics and electronic boards. When warmed, these materials can leak TBBPA to the environment with potentially dangerous consequences for human health; e.g. the compound has been found in the blood of workers at an electronic products recycling plant [1]. TBBPA is ubiquitous in the environment, having even been discovered bound to dust particles in air, in river sediment and mussels [2]. Although the World Health Organization (WHO) [3] and the European Scientific Committee on Health and Environmental Risks (SCHER) [4] concluded that TBBPA did not present risks to human health, scientific studies have demonstrated a potential hazard of these compounds [5]. Moreover, TBBPA has been classified as very toxic to aquatic species. The purposes of our study were to implement an innovative solution for liquid chromatography-mass tandem (LC-MS/MS)-based analysis of TBBPA in fish and bivalves and to apply this novel method for examination of different fish species and bivalves collected from different European regions within a monitoring survey organized in the framework of the ECsafeSEAFOOD project [6]. Sample preparation comprised liquid–liquid partitioning with acetonitrile in presence of anhydrous $MgSO_4$ and NaCl (QuEChERS). The ACN-extracts were added with water followed by extraction with (a) hexane/MTBE and (b) benzene/MTBE. The final extracts were dried, concentrated and redissolved in 1 mL MeOH and transferred to a vial for LC–MS/MS analysis. Good performance characteristics were achieved during validation of the method for both fish muscle tissue and bivalves. Recoveries ranged from 65 to 86%. A good linearity and precision, with relative standard deviations generally below 20% were obtained. Method limits of quantification were in the range 20–100 ng/g dry weight. The method was used for analysis of 21 fish and 5 bivalve samples. TBBPA was detected in four fish samples and one bivalve sample. The results obtained were in accordance with those from similar studies published worldwide.

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Keywords: *Tetrabromobisphenol A, LC-MS/MS, QuEChERS, Fish, Bivalves*

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F24
MYTILUS GALLOPROVINCIALIS AS INDICATOR OF PBDES CONTAMINATION IN MARCHE REGION (ITALY): EXTENSIVE MONITORING RESULTS.

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Polybrominated diphenyl ethers (PBDEs) are a class of Brominated Flame Retardants (BFRs) used worldwide as additives to inhibit or slow down the ignition of fire in various consumer products. They are persistent and have very low water solubility, high binding affinity to particles and tendency to accumulate in the environment and biota. Accordingly in 2008 the European Union banned their use and in 2010 PBDEs were included in the Stockholm Convention POPs List. In Italy very little has been done to study the environmental levels and dietary exposure, therefore there is the need to gain knowledge on PBDEs contamination patterns and levels. This study involved the analysis of fifteen PBDE congeners (28, 47, 49, 66, 77, 85, 99, 100, 138, 153, 154, 183, 197, 206, 209) in 134 mussel samples collected from April till November 2013 in 21 breedings or reefs located along the coast of Marche region (Middle Adriatic Sea). Bivalves accumulate high concentrations of various toxic pollutants and they are commonly used as pollution indicators in the marine environment. The choice of mussels as sentinel organisms enables suitable comparison with the many available data for the same species in Europe. The samples were analysed using an in-house method which involves a QuEChERS-like extraction followed by a two steps clean-up and a GC triple-quad MS/MS analysis using isotopic dilution for quantification purpouse. Only four congeners were detected: BDE-49 (mean=15, min=5.0, max=42 pg/g), BDE-47 (mean=73 min=16 max=186 pg/g), BDE-100 (mean=18, min=4.1, max=68 pg/g) and BDE-99 (mean=30, min=4.4, max =91 pg/g). The other eleven PBDEs were below the method LOQs. The concentrations of PBDEs (sum) ranged from 27 to 386 pg/g. These results are in agreement with the data published by Bianco et al. (2010) and Giandomenico et al. (2013) on Apulia mussels. Also Bianco et al. reported 47, 99 and 100 as predominant congeners; they didn't analyze the BDE-49. Generally PBDEs levels measured in North America and Asia are much higher than those found in mussels from Adriatic Sea. The average concentrations of the PBDEs sum measured in each of the 21 sampling stations were studied grouping them as a function of their geographical location along the coastal area. The highest levels were measured around Ancona and the concentrations in this area were significantly higher with respect to the observations mad e in all the other stations ($p < 0.01$). This was expected because an oil refinery is located northern of Ancona and Ancona itself is the most urbanized area of the region and its harbor is one of the most important of the Adriatic Sea with tourist and commercial activities. Further researches using the same organism as indicator should be implemented to evaluate and compare PBDEs contamination in other Italian coastal areas.

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Keywords: *Polybrominated Diphenyl Ethers, Mussels, Sentinel organisms, POP, Brominated Flame Retardants*

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F25
MULTI-CLASS ANALYSIS OF POLYCHLORINATED BIPHENYLS, POLYCYCLIC AROMATIC HYDROCARBONS, POLYBROMINATED BIPHENYL ETHERS AND ORGANOCHLORINE PESTICIDES IN ANIMAL FEED

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The number of organic contaminants introduced in the environment and potentially dangerous for human health is continuously increasing and therefore constantly growing is the number of analytes to be controlled by official laboratories in food and feed. The aim of the present work was to develop a single multi-class method to be used for the simultaneous monitoring of six non-dioxin-like (NDL) indicator PCBs, twenty-three persistent OC pesticides, four priority PAHs and ten Polybrominated Diphenyl Ethers (PBDEs) in animal feed. Here we report the optimized instrumental conditions and the preliminary results of the sample preparation procedure adopted for the analysis of the above mentioned forty-three organic pollutants using a gas-chromatograph coupled to triple quadrupole mass spectrometer (GC–MS/MS). In the method development a capillary column was selected fulfilling the following criteria: elution without degradation of the heavy BDE209, separation of a II the isobaric molecules, resolution of the six indicator PCBs from other interfering congeners (28 from 31, 52 from 69, 138 from 163/164) and resolution of the three fluoranthene isomers (Benzo(b)-, Benzo(k)- and Benzo(j)-fluoranthenes). The best results were obtained with Agilent Technologies DB-XLB (15m × 180 µm x 0.07 µm). In order to maximize the method sensitivity, 10 microL of sample extract were injected in Programmable Temperature Vaporization (PTV) mode. Dwell times were selected and acquisition windows adjusted to maximize the acquisition frequency. Two transitions for each analyte were acquired, three in case of pseudo-transitions (parent-parent ion) or in case of two precursor or product ions belonging to the same isotopic cluster. The ionization energies were also optimized. Isotope dilution quantification technique was adopted for PCBs, PAHs and most of the PBDEs, while in case of pesticides the internal standard approach was the best choice. Eight grams of sample were extracted by QuEChERS ($MgSO_4$, NaCl, H_2O and ethyl acetate) and then different type of clean-up procedure were tested: different SPE stationary phases, GPC and dispersive SPE. The best results were obtained with dispersive z-sep SPE (500 mg) in acetonitrile combined with a subsequent Si-SPE (2 g /6 mL). The results seem encouraging, but still some optimization has to be done and then an exhaustive validation of the method should be performed. Surely the possibility to simultaneously analyse forty-three POPs in a single method is a very interesting result enabling a better monitoring of those ubiquitous contaminants. Official laboratories should be encouraged to adopt multi-class methods to guarantee a more exhaustive control of residues in food and feed.

[1] Regulation EU/277/2012; Regulation EU/835/2011; Directive 2002/32/CE and amendments; Recommendation EU/118/2014.
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Keywords: *GC-QQQ MS/MS, z-sep dispersive SPE, Multi-class, POPs, QuEChERS*

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F26
ANALYTICAL STRATEGIES FOR THE MONITORING OF POLYBROMINATED DIPHENYL ETHERS IN FOOD

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Brominated flame retardants (BFRs) are chemical mixtures which are applied to products such as plastics and synthetic fibers in order to inhibit or slow down the ignition in case of fire. It has been shown that BFRs are persistent, toxic and bioaccumulative and they have been found in environment due to leaching from the products in which they were used. As a consequence, these substances have also over time reached the food chain.

The European Commission published in 2014 a Recommendation on the monitoring of traces of BFRs in foodstuff (2014/118/EU). The Recommendation established limits of quantification (LOQ) for the different groups of BFRs (polybrominated diphenyl ethers, hexabromocyclododecanes, tetrabromobisphenol A and derivatives, brominated phenols and derivatives, emerging and novel BFR) in order to detect them in a wide variety of food commodities.

Following the legislation, for the class of polybrominated diphenyl ethers (PBDEs), up to ten congeners (BDE-28, 47, 49, 99, 100, 138, 153, 154, 183 and 209) should be analyzed in different food commodities. These commodities include: eggs and egg products, milk and dairy products, meat and meat products, animal and vegetable fats and oils, fish and other seafood, products for specific nutritional uses and food for infants and small children. The limit of quantification of the analytical methods should be 0,01 ng/g wet weight or lower.

As it is well known, the analysis of PBDEs, especially of the highly brominated congeners is a real challenge for many laboratories and the high sensitivity necessary to analyze these compounds is not easily achievable. Of special concern is the analysis of BDE-209 and samples with high fat content.

The Laboratori de l'Agència de Salut Pública (LASPB) has analyzed PBDEs in fish and seafood, as part of the official control programs since 2009. The method is currently included in the scope of the accreditation of the laboratory following ISO/IEC 17025 requirements. However, new congeners have been included and a special effort has been done in order to get lower LOQs to fulfill the Recommendation. For this purpose, an extraction method based on QuEchERS technology has been optimized, modifying the solvent extraction and clean-up conditions, and developing a new instrumental GC–EI—MS/MS method to achieve the LOQs required.

Moreover, in order to study limitations and advantages of the method developed in LASPB, the extracts of the same samples have been analyzed using a GC–EI–HRMS instrument and a GC–APCI–MS/MS instrument. Results have been compared and conclusions about GC–HRMS and soft ionization sources have been obtained.

Keywords: *brominated flame retardants, food commodities, GC-EI-HRMS, GC–APCI–MS/MS, Recommendation 2014 /118/EU*

F27
VALIDATION METHOD FOR THE DETERMINATION OF UV FILTERS IN RIVER MUSSELS FROM THE DANUBE BY LC-ESI-MS/MS ANALYSIS

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UV filters constitute a heterogeneous group of chemicals used to protect against the harmful effects of UV solar radiation. Recent increasing interest in the adverse impact on human health by excessive sunlight exposure has resulted in the widespread use of organic UV filters. In the last few years at the top of public interest there are secondary effects of these compounds, because their ingredients may enter the aquatic environment and reach detectable and harmful concentrations. The recent experimental studies indicate that several UV-filters might have endocrine disruptive effects. Also, these compounds can have possible adverse effects on the developing organs of fetuses and children. Taking into consideration the fact that the river mussels (Unio pictorum) are unique specialties of local population, the method validation for the determination of four UV filters (benzophenone-3, octocrylene, 4-methylbenzylidencamphe and octyl methoxycinnamate) was done. The method involves modified QuEChES method for the extraction of UV filters. The extracts were analysed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI–MS/MS). The methodology allowed for the determination of target analytes at trace levels. LOD was calculated by MassHunter Qualitative Software and it was below 3.0 µg/kg. The linearity was checked using matrix matched calibration from 5.0 to 50 ng/mL with the R^2>0.99 for all investigated UV filters. The recovery data obtained by spiking river mussel samples at two concentration levels (10 and 50 µg/kg) was in the range from 89.8–115.2±5.7–11.96%. The relative standard deviation was below 20% for all target analytes.

Keywords: *UV filters, river mussels, QuEChERS, LC–ESI–MS/MS*

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F28
SHOOT-AND-DILUTE GAS CHROMATOGRAPHY-MASS SPECTROMETRY: POLYCYCLIC AROMATIC HYDROCARBONS QUANTIFICATION IN TEA USING MODIFIED QUECHERS EXTRACTION AND NO SAMPLE CLEANUP

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Food contamination with toxic polycyclic aromatic hydrocarbons (PAHs) occurs by exposure to environmental contamination and during food preparation, especially heat processing like smoking, grilling and roasting. Consequently, foods must be tested to determine if they contain toxic PAHs. While classic sample extraction methods such as Soxhlet and Pressurized Fluid Extraction (PFE) yield excellent recoveries for PAHs, they require expensive equipment/glassware, are solvent- and resource-intensive, and are relatively slow. The QuEChERS sample preparation method avoids these problems but produces a comparatively dirty sample. There are notorious problems associated with splitless injection of dirty samples, most notably for PAHs is decreasing response. This can occur very quickly with real samples, especially without exhaustive sample cleanup. Methods to address these problems explored here included streamlined sample preparation and Shoot-and-Dilute gas chromatography-mass spectrometry (GC–MS). A modified QuEChERS extraction was paired with a simple silica solid-phase extraction (SPE) cleanup. This sample preparation method is much less resource intensive and provided satisfactory recovery of all PAHs tested. In addition, sample extracts without cleanup were also analyzed in order to determine if sample cleanup was necessary when combined with Shoot-and-Dilute GC–MS/MS. Shoot-and-Dilute GC–MS/MS methods used split injection which can alleviate matrix related issues occurring at the GC inlet and column. Increased flow through the inlet during split injection minimizes poor response for involatile compounds (e.g. high molecular weight PAHs) and maintains acceptable data quality longer. This work demonstrates that the ruggedness and sensitivity of Shoot-and-Dilute GC–MS/MS allows quantitation of the EU 15+1 PAHs in extracted tea samples without the need for sample cleanup. Incurred values at sub 10 ng/g levels were determined and proved similar to values determined via splitless injection GC–TOFMS. Ruggedness was evaluated by tracking PAH relative response factors for over 100 injections of tea extracts with no cleanup. Even with no inlet or column maintenance, response factors were consistent (less than 20% RSD) even for the highly nonvolatile dibenzopyrenes. The combination of split injection and highly sensitive GC–MS/MS allowed samples with low PAH levels to be quantified without sample cleanup and, at the same time, prolonged system performance.

Keywords: *polycyclic aromatic hydrocarbons, shoot-and-dilute, GC–MS/MS, modified QuEChERS, tea*

F29
QUANTITATIVE DETERMINATION OF 32 FORBIDDEN DYES IN FOOD PRODUCTS WITH LC–ESI–MS/MS

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Synthetic dyes are widely used in food products. A large number of these dyes, for example Sudan dyes, are not allowed in food stuff in the European Union. Nevertheless, ppb levels of forbidden dyes are regularly reported today. Therfore is a great need for efficient methods of analysis for these compounds [1,2]. Here a method approach is presented for the accurate quantitative determination of 32 dyes in food stuff with LC–ESI–MS/MS. Due to the large variability in polarity (21 moderate polar to non-polar compounds, 11 highly polar compounds), a method approach with two different mobile phase systems but with the same LC column was developed. For all dyes with exception of cis-bixin three mass transitions were acquired in each case (for cis-bixin six mass transitions). The evaluation of analytes was carried out using calibration standards of defined concentrations. To take into account losses during sample preparation and ion suppression effects, all results were corrected via external recovery of spiked recovery samples. An exception was Sudan I for which a correction was carried out via internal recovery due to the availability of an internal Standard (D5-Sudan I). Validation of the method for all 21 moderately polar to non-polar compounds shows linearity in the range of 1 to 125 µg/L. For the 11 highly polar dyes the linearity range was 5 to 625 µg/L. For all analytes, the value of R^2 for linearity was ≥0.995 and therefore acceptable. The precision for matrix-free as well as matrix-matched sample solutions was good as well and shows values for coefficient of variation of ≤ 10%. For spices and oleoresins, the LOQ´s for the 21 moderately polar to non-polar compounds were in the range of 10 µg/kg, for most analytes. For the 11 highly polar compounds the LOQ´s were in the range of 50 µg/kg, due to ion suppression caused by the mobile phase.

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Keywords: *forbidden dyes, LC-MS/MS, spices, oleoresins, food contaminants*

F30
THE DETERMINATION OF ARSENIC SPECIES IN RICE BY A RELIABLE METHOD SUITABLE FOR ROUTINE QUALITY CONTROL

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Arsenic as trace element occurs ubiquitous in the environment. Therefore, arsenic in foodstuff is an important aspect of food safety. Rice as an arsenic accumulating plant often contains considerable levels of inorganic arsenic. It has to be distinguish between the single arsenic species as they are different in regard to their toxicology. The inorganic forms (As (III) and As (V)) are classified as human carcinogen, whereas the organic forms (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) are classified as toxicologically harmless. As a consequence, in January 2016 maximum levels for inorganic arsenic in rice and rice products will be set by the Regulation (EC) No. 1881/2006. Diverse methods for the determination of inorganic arsenic are either complex or often not routine suitable or the methods are simple but not reliable. For the complex methods, an inert gradient LC (anion-exchange)-system is required, while the simple methods (e.g. extra ction of the inorganic arsenic by hydrochloric acid; analysis by ICP–MS) provide inaccurate results. Consequently, a reliable method for the determination of inorganic arsenic, suitable for high-throughput routine quality control, is required. The presented method provides good validation data, a rapid analysis time and is easy to perform. Thus, the method is perfect suitable for routine quality control in a high-throughput laboratory.

Keywords: *inorganic arsenic, LC-ICP-MS, rice, arsenic species, routine-analysis*

F31
A HIGHLY SELECTIVE AND SENSITIVE LC-MS/MS METHOD FOR THE ANALYSIS OF THE BACTERIAL TOXIN CEREULIDE IN FOOD

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Cereulide is a bacterial toxin produced by some Bacillus cereus strains under certain conditions, e.g. storage of cooked meals at insufficient temperatures. The consumption of cereulide contaminated food usually causes nausea or emesis, but can also become severe or even fatal in rare cases. Due to its cyclic structure with alternating ester and amid bonds, cereulide is highly resistant to heat and retains its toxic activity, even after Bacillus cereus has been inactivated. Thus, it is of great importance to not only perform a microbiological analysis but also determine cereulide in (suspect) food. The present contribution describes the development and validation of a highly selective, sensitive and robust LC–MS/MS method for the analysis of cereulide in various complex foods. $^{13}C_6$-cereulide was used as internal standard to achieve optimal and robust quantitative results by compensating for extraction recoveries and possible matrix effects. The mass spectrometric behaviour of cereulide (formation and fragmentation of different adducts, optimisation of fragmentation parameters, etc.) and the optimisation of the HPLC conditions (mobile phase additives, column temperature, etc.) will be presented. Sample preparation was based on the QuEChERs method, yielding pure extracts with a minimum amount of work. Due to the highly selective MRM transitions very low limits of detection and quantification of 0.06 and 0.2 µg/kg, respectively, were obtained. The LC–MS/MS method was validated using cooked rice. Recovery rates ≥84% and good intermediate precision with an RSD ≤16% over the whole spiking range were achieved. The method was employed for the analysis of food samples from the official Austrian food control and found to be suitable for the rapid analysis of cereulide in various complex food matrices such as meat filling, fried chicken, pumpkin soup or cream cake. The obtained results for cereulide will be presented and compared to the amounts of Bacillus cereus found in these samples.

Keywords: *cereulide, bacterial toxin, Bacillus cereus, LC–MS/MS, validation*

F32
A SOFTWARE-HARDWARE SYSTEM FOR DETECTION AND IDENTIFICATION OF PATHOGENS

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We describe a new technology that has been developed for rapid detection and subsequent identification of bacterial colonies. The system relies upon the principle of measurement of elastic light scatter (ELS) using a laser beam to impinge upon a bacterial colony on an agar plate. The elastic scatter pattern produced by the complex association of bacteria in the colony produces a complex scatter pattern directly onto a CCD array immediately beneath the petri dish. We have developed a software-hardware combination that locates the colony, moves the laser beam to each colony and collects the "colony fingerprint" and records this information. The software package is a complex system for management of the movement of the petri disc, finding the colonies and creating a management system to record all the data. Using an associated software package, it is possible to analyze each colony fingerprint and develop classifiers that can be used for future analysis. This paper will outline the system operation and management of the entire process from finding colonies to the classification and identification.

Keywords: *software, microbial detection, laser based analysis, classification of organisms*

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F33
ANALYSIS OF SELECTED POPS WITH AN ATMOSPHERIC PRESSURE CHEMICAL IONISATION GC COUPLED TO HIGH-RESOLUTION QTOF MS

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Trace analysis of polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF) and polybrominated diphenyl ethers (PBDE) is one of the challenges in analytical chemistry. As they belong to the class of persistent organic pollutants (POPs) they are one of the major concerns in present environmental discussion. Due to the accumulation in the food chain it is of general interest to analyze them with good sensitivity and confidence.
GC-APCI coupled to a high resolution QTOF–MS offers a suitable and sensitive analytical tool for the analysis of those POPs. Here we report a method for Decabromodiphenyl ether (DecaBDE) and 2,3,7,8-Tetrachlordibenzodioxin (2,3,7,8-TeCDD) using a Bruker 450-GC coupled to an impact II (Bruker Daltonik GmbH) as they are key substances for their compound classes. Quantitation was done in fullscan mode.
PBDEs are among the EU priority substances. DecaBDE is the most difficult PBDE to analyze, because it is less volatile and additionally thermolabile. DecaBDE showed a good response at a concentration of 1 pg on column, LOD was even lower. The analytical working range was between 1–40 pg on column.
2,3,7,8-T_4CDD is the most toxic substance of the PCDD/PCDF. 2,3,7,8-T4CDD was detected as $[M]^+$ signal, LOD of 2,3,7,8-T4CDD was <0.1 pg on column. The calibration curve showed an analytical working range between 0.1–2000 pg on column. Applying a higher collision energy caused a loss of COCl that could be used for confirmation.
Using GC–APCI coupled to high resolution QTOF–MS we achieved excellent detection limits at relevant environmental limit values.

Keywords: *QTOF–MS, GC–APCI, persistent organic pollutants*

F34
ASSESSMENT OF BENZENE, BENZOIC ACID AND ASCORBIC ACID IN JUICE DRINKS IN NAIROBI COUNTY, KENYA

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Background Empirical evidence has established that sodium benzoate degrades to generate traces of benzene in the presence of ascorbic acid. The rate of degradation is also dependent on the pH, exposure to UV light and presence of metals such as copper and iron. Benzene is classified as carcinogenic to humans.
Objective The study examined the possible formation of benzene under differing storage conditions in market outlets from sodium benzoate used as a preservative in water-based flavoured fruit juices, and in some cases fortified with ascorbic acid.
Methods The pH, benzene, benzoic acid, and ascorbic acid were studied from 15 juice samples each from kiosks and open air market, respectively, and 30 from supermarkets. pH meter and HPLC with a UV visible detector were used with standard and validated methods.
Results Kiosk samples had the highest amounts of benzoic acid (311.82±179.29) while open market had the lowest amounts (166.04±64.67 ppm). Benzoic acid was significantly higher in the samples from kiosks compared to those from open market (P=0.006), and supermarket (P=0.01). Similarly, benzoic acid from open market and supermarket samples were significantly different (P=0.003). Variation in the amounts of benzoic acid was highest amongst samples from the kiosks, followed by the open market. Supermarket samples had the most consistent amounts of benzoic acid (226.50±18.81 ppm). There was no detectable benzene from samples from supermarket, while 26.7% (n=4) from open market had benzene which ranged from 2.71 to 21.17 ppb, and one sample from kiosks with 4.55 ppb. The prevalence of benzene is 8.33% in Nairobi Central Business District (NCBD).When the juices were stratified by flavor, only the orange flavor had benzene. For ascorbic acid, 5% (n=3) had detectable levels that ranged from 33.22 to 171.02 ppm and these were two from kiosks and one from the supermarkets. There was no association for the amounts of ascorbic acid to benzene. A comparison of the pH of the juices showed that those from open market had a significantly higher pH than those from kiosks (P=0.002), while the open air and supermarkets were comparable (P=0.06). The pH between kiosks and supermarket was significantly different (P=0.03). The observed low level of benzoic acid in samples from open market could be attributed to degradation due to high temperature and exposure to UV light, and would the contributing factor to the observed amounts of benzene in the four (4) samples. This is demonstrated by the strong negative correlation (-0.576) for the benzoic acid and benzene in samples from open market and the high pH observed. Generally for all the 60 samples the amounts of benzene appear to negatively correlate with the levels of benzoic acid (-0.667).

Keywords: *ascorbic acid, benzene, benzoic acid, pH, fruit-flavoured juice drinks*

F35
EVALUATION OF MEASUREMENT UNCERTAINTY FOR PERSITENT ORGANIC POLLUTANTS ANALYZED BY ISOTOPE-DILUTION MASS SPECTROMETRY

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In the context of EU legislation where maximum for contaminants were set for various kinds of food and feeding stuffs, measurement uncertainty (MU) is used for compliance assessment. A survey within the European network of National Reference Laboratories (NRLs) pointed out large discrepancies of MU reported, depending on the approach used by laboratories. In 2012, a working group on MU within the network of EU-RL and NRLs for POPs was established. The main task was to evaluate a harmonized approach for estimation of MU for POPs based on measurement by isotope dilution mass spectrometry.
International bodies, ISO or Eurachem, recognize inter-laboratory study and validation processes as a valid basis for MU in analytical work. Both sources are appropriate to ultra-trace analysis of POPs, mainly due to the complex sample preparation and measurement steps required. The main disadvantage of this approach is that it relies on data that has been recorded during a validation study and the MU calculated does not necessarily reflect the uncertainty associated with results obtained in daily routine analyses. In addition, it gives little insight as to where the major sources of uncertainty lie because it is based on a global (i.e. top-down) approach. In order to overcome the drawbacks highlighted, an on-going top-down approach is suggested that integrates historical data, reflecting the precision and trueness of the analytical method but also additional tools such as matrix-based quality control (QC), PTs results generated several times a year, the daily limits of quantification (LOQs), matrix and procedural blank effects, and the variability of relative response factors (RRFs) in order to take into account as much as possible the impact of daily performance in the MU assessment.
For precision studies, the experimental design consists of a long-term precision study for looking at repeatability and between run variability by using naturally contaminated quality control (QC) samples. The QC samples must match matrix and levels of interest. The total range will cover 0.5x the lowest maximum limit, to 2x the highest maximum limit. The trueness study, based on relevant matrix CRMs, or matrix-matched spiking experiments, and inter-laboratory studies or PTs. Participation in PTs is a good alternative in order to cover a full range of food and feed matrices for trueness assessment. In order to rationalize the diversity in matrices, the working group decided to differentiate matrices for PTs according to the method of analysis (e.g. fat based matrices, feed, dry food,…) and suggests 6 participations in PTs resulting on average in a moving time window of 3 years if laboratories participate twice a year at PTs.
The working group will continue to consolidate the above findings and use examples of real data to finalize the approaches to estimating MU associated with the determination of POPs and other contaminants analyzed by isotope dilution mass spectrometry.

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[2] ISO/TS 21748:2004(E), Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation.

Keywords: *measurement uncertainty, contaminants, POPs, isotope dilution, mass spectrometry*

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F36
FAECAL SOURCE TRACKING IN SWEDISH RAW WATER BY USING A UNIQUE COMBINATION OF CHEMICAL MARKERS AND APCI–ESI–LC–MS/MS

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Drinking water contaminated with faeces poses health risks in urban and rural areas around the world. In Sweden drinking water is regulated by the National Food Agency. During winter 2010–2011, when nearly 40,000 people allocated in two areas in the north of Sweden got ill from drinking water infected with Cryptosporidium homunis, a parasitic protist living in human faeces, the NFA had no quick tool to track the contamination. The method presented aims at tracking the fecal source of contamination which allows authorities to locate and remove sources more efficiently. We present a fast versatile LC–MS/MS method for the analysis of water such as influent, effluent, surface- and drinking water. A scope of 50 substances was chosen based on their suitability as species specific (fecal) marker, for example, monensin and narasin (coccidiostats, chicken/turkey marker), sterol metabolites (all species, different ratio's), fluorescent brightener (human) but also be nzo-a-pyrene (urban stormwater marker). These compounds were extracted from water with an automated large volume extractor (SPE–DEX®). For the MS method two distinct ionization techniques are applied, ESI (electrospray ionization) and APCI (atmospheric pressure chemical ionization) so that both polar/moderate polar (log Kow < 5) and non-polar (log Kow >7) substances can be analyzed. The overall recovery was between 10–110% and the reproducibility between 2–37%, the LOD were between 1–15 ng/l and 1–15 µg/l for the sterol metabolites. The method was tested with an in-house proficiency test, where different water types were spiked with faeces from different species. This PT proved the applicability of the presented method and showed that especially the sterol metabolites play a conclusive role in determining the source of fecal contamination. By calculating the ratio of certain sterol metabolites, the origin of the faeces is easily determined and a distinction can b e made between fecal contamination from human, herbivore, pig or chicken. Also, samples were analyzed from an agricultural area in the south of Sweden. The sampling was designed to locate source(s) of contamination present in that specific drinking water area. Raw water samples were for example taken in close proximity to farms and other places with anthropogenic activity. Remarkable was the fact that the method detected sterol metabolites in the water since these compounds mostly bind to small particles and thus end up in the sediment due to their low solubility (logD 7.5). Meaning, this sensitive method avoids an additional extraction of sediment. Conclusively, this fecal source tracking method is fast and sensitive and capable of detecting all included chemical markers in raw water. Especially the sterol metabolites have shown their usefulness as a tool for feacal source tracking and should thus be used by the community to give information about the quality of their drink ing water in regard to feacal contamination.

Keywords: *sterol metabolites, APCI, fecal source tracking, pharmaceuticals, fluorescent brightener*

Acknowledgement: *Swedish Civil Contingencies Agency*

F37
IDENTIFICATION AND DETERMINATION OF 413 TOXICANTS OF DIFFERENT CLASSES BY LC-ESI-QTOF IN FOOD PRODUCTS, FOOD RAW MATERIALS AND FEED

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The food products of plant and animal origin, feed and food raw materials may contain tens or hundreds of toxicants of different classes: mycotoxins, pesticides and veterinary drug residues, various additives and so on. Nowadays identification and determination of these toxicants from single sample with minimal effort are urgent problem of analytical chemistry of food and feed. The methodology of identification and determination of 413 toxicants of different classes in food and raw materials for food industry from single sample by the LC–ESI–QTOF combined with high performance liquid chromatography using a quick, simple and easy sample preparation have been proposed. Analytes are pesticides of different classes, mycotoxins, veterinary drugs and coloring matter for foodstuffs. Simple sample preparation variations for milk, meat, fat, eggs, liver, kidney, feed and grain were suggested. Sample preparation involved the extraction with acetonitrile and removal of fat by extraction with hexane. Under the electrospray ionization conditions most analytes transform to protonated and deprotonated forms and rarely found adducts containing ammonium, sodium or potassium. Identification of analytes was carried out using the «TargetAnalysis-1.3» program. Retention time, the *m/z* of one fragment ion (because of HR MS was using) and isotope distribution match (mSig ma) were used as identification parameters. Low limits of detection of analytes were shown to be 0.0005–50 ng/ml. It was found that, given such low LoD, the dilution of the extract with water to eliminate the matrix effect is possible. The lowest calibration level considering the sample preparation and dilution amounted to 1 (500) µg/kg. The recovery of analytes from the analyzed samples ranged from 78 to 110% depending on the nature of the analyte and the matrix. A scheme for the identification and determination of contaminants by standard addition was proposed. The advantages of the standard addition method compared to the method of the calibration curve in the determination of toxicants in real samples were demonstrated. The relative standard deviation of the results does not exceed 0.1. The identification period was 40–60 min, and the determination of identified toxicants was in the range of 2-3 hours.

Keywords: *LC–ESI–QTOF, food & feed, mycotoxins, pesticides, veterinary drugs*

F38
QUANTITATIVE ANALYSIS OF STAPHYLOCOCCAL ENTEROTOXINS A AND B IN FOOD MATRICES USING ULTRA HIGH−PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (UPLC–MS/MS)

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A method that uses mass spectrometry (MS) for identification and quantification of protein toxins, staphylococcal enterotoxins A and B (SEA and SEB) in milk and shrimp is described. The analysis was performed using a tryptic peptide, from each of the toxins, as the target analyte together with the corresponding ^{13}C-labeled synthetic internal standard peptide. The performance of the method was evaluated by analyzing spiked samples in the quantification range 2.5–30 ng/g (R^2=0.92–0.99). The limit of quantification (LOQ) in milk and the limit of detection (LOD) in shrimp was 2.5 ng/g, for SEA and SEB toxins. The in-house reproducibility (RSD) was 8–30% and 5–41% at different concentrations for milk and shrimp, respectively. The method was compared to the ELISA method, used at the EU-RL (France), for milk samples spiked with SEA at the low levels in the quantification range, 2.5 and 5 ng/g. The comparison showed good coherence for the two methods: 2.9 (MS)/1.8 (ELISA) and 3.6 (MS)/3.8 (ELISA) ng/g. The major advantage of the developed method is that it allows direct confirmation of the molecular identity and quantitative analysis of SEA and SEB at low nanogram level using a label and antibody free approach. Therefore, this method is an important step in the development of alternatives to the immuno−assay tests currently used for staphylococcal enterotoxin analysis.

Keywords: *staphylococcal enterotoxins, quantification, foods, UPLC–ESI–MS/MS*

F39
RISK MANAGEMENT: PRIORITY TESTING AND FUTURE HAZARD ANALYSIS

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Food Safety, Quality and Integrity are foundations for any reputable food company and any issues which undermine these principles have the potential not only to cause harm to the consumer but also catastrophic damage to the company involved and the entire food industry. Therefore, an accurate risk assessment of ingredients and supply chain is required. A Knowledge Transfer Partnership (KTP) project has been undertaken by Moy Park Ltd., one of the UK and Europe's leading high quality food companies, in cooperation with the Institute for Global Food Security (IGFS) at Queen's University Belfast, aiming to prioritise possible food contaminant risks. Goals of the project are: (i) to develop a risk management model that can be used to rank identified hazards in terms of importance for analytical testing; (ii) to identify and review future testing methods to be possibly transferred into Moy Park's own laboratories. IGFS previously developed a unique risk identification and management system (entitled FeedRisk-Select) for the animal feed industry on the island of Ireland. The KTP project has used the same approach, in conjunction with systems developed previously in-house by Moy Park Ltd., to create a similar risk identification. Details on raw materials/food ingredients used by Moy Park Ltd. have been collected on a confidential basis. Using the data available in the EU RASFF (Rapid Alerts System for Food and Feed), hazards associated with each food ingredients category have been identified. A risk scoring system has then been calculated, focusing on frequency of hazard occurrence and hazard severity, and testing priorities for all the Moy Park food ingredients have been identified. Based on the main priority testing results, a review is currently being undertaken to understand the types of analytical and bioanalytical methods which are available to detect known hazards in a fast, reliable and cost-effective manner.

Keywords: *risk, hazard, ingredients, food*

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F40
METHOD OF PARALLEL DETERMINATION OF BROMINATED AND CHLORINATED DIOXINS IN FOOD

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Persistent organic pollutants (POPs) have adverse effects on the health of humans and wildlife. Among the POPs, dioxins and dioxin-related compounds such as well known polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) and little known polibrominated dibenzo-p-dioxins/dibenzofurans (PBDD/Fs) have similar physicochemical properties, exert toxic effect to humans and they work by the same mechanism of toxicity via Ah receptor. Therefore, from the point of view food safety and human health protection data concerning PBDD and PBDF levels in food is important information. Methods of determination of PCDD/F and PCBs in food are regulated under the EU legislation, but do not comprise the other related compounds. The aim of the study was to develop parallel determination of brominated and chlorinated dioxins in the same purified extract samples using isotope dilution method (IDMS) with HRGC/HRMS congener identification and to assess its suitability for routine use. The ^{13}C labelled standard of 4,2,6,8 TBDF was used for recovery and ^{13}C 1,2,3,4-TCDD for checking the possible UV degradation process of ^{13}C brominated standards and corresponding compounds. UV light protection during the sample cleanup and concentration was applied. Instrumental detection and quantification of chlorinated and brominated dioxin and furans was made in the same extract during two separate GC runs. Limits of quantifications were congener dependent and ranged from 0.01 to 0.16 pg/g of fresh weight for fish tissues, and from 0.07 to 1.42 pg/g of fat for hen eggs. Labelled standards recoveries was in the range of 50 to 143% with exception of di-, tri- and octa brominated congeners for lower values. Total li mits of quantification was 0.04 pg WHO-PBDD/F-TEQ/g fresh weight for fish and 0.33 pg WHO-PBDD/F-TEQ/g fat for eggs. The above methods parameters are comparable with analytical criteria for chlorinated dioxins described in EU Commission Regulation No 589/2014. Statistically significant differences was observed (Mann-Whitney U-Test, p=0.05) for tetra- and penta- chlorinated vs. brominated compounds recoveries. The applied methodology allows for parallel determination of 29 PCDD/F/DL-PCB congeners, 6 NDL-PCBs and 16 polibrominated dibenzo-p-dioxin/furans congeners (2,3,7-TriBDD, 2,3,7,8-TeBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8-HxBDD, 1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, 1,2,3,4,6,7,8-HpBDD, OBDD, 2,8-DiBDF, 2,4,8-TriBDF, 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF, OBDF). Taking into account the recoveries and quantification limits, the method can be used for parallel determination of brominated and chlorinated dioxins and furans in fish and eggs, in which brominated dioxins can be mostly expected.

Keywords: *PBDD/Fs, GC–HRMS, fish, eggs, method of determination*

F41
A NEW MULTI-CLASS METHOD FOR DETERMINATION OF HALOGENATED CONTAMINANTS IN HUMAN SERUM

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Nowadays, the biomonitoring of various groups of contaminants including persistent organic pollutants (POPs) is a very important issue since humans are exposed from the environment as well as diet to a cocktail of chemicals. Most of these substances have potential negative effects on the organism including potential carcinogenity, neurotoxicity and many of them can act as endocrine disruptor. One of the mostly used biological indicator for the monitoring of POPs levels represented by polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), brominated flame retardants (BFRs) and per- and polyfluoroalkylated substances (PFASs) in the population is human serum. In general, within these biomonitoring studies only a limited amount of sample (1−5 mL) for the analysis is usually available. From this reason the main aim of the present study was to develop and validate an analytical multi-class method for the simultaneous determination of a wide range of halogenated contaminants, namely PCBs (n=8), OCPs (n=11), PFASs (n=19) and BFRs (n=31), when only 3 mL of blood serum is required. For the identification/quantification of target analytes both instrumental techniques, liquid chromatography (LC) and gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS), were used. The tested sample preparation procedure was consisted from several steps. In the first phase the polar solvent (acetonitrile) was added to the sample. After that a multiple extraction (three times) using relatively nonpolar extraction mixture (*n*-hexane:diethylether, 9:1, *v/v*) was used for the isolation of PCBs, OCPs and BFRs including polybrominated diphenyl ether s (PBDEs) and hexabromocyclododecanes (HBCDs). The upper nonpolar fraction was purificated on silica column and final extract was analyzed by GC−MS/MS with electron ionization (EI) for PCBs and OCPs and negative chemical ionization (NCI) for PBDEs and non-PBDEs. The lower polar layer was once again extracted by QuEChERS based approach and isolated PFASs including several BFRs were analyzed by LC−MS/MS. The recoveries (n=6) obtained within the validation experiments were in the range of 57−130% and 76–122% for GC and LC amenable compounds, respectively. Repeatabilities (expressed as relative standard deviation) were lower than 20 %. The limits of quantifications were in the range of 0.5 –10 ng/g lipid and 0.01–0.33 ng/mL for GC and LC amenable compounds, respectively.

Keywords: *serum, POPs, PFAS, BFR, LC–MS, GC–MS*

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F42
HIGHLY SENSITIVE AND SELECTIVE QUANTIFICATION OF MICROCYSTIN TOXINS IN DRINKING WATER BY UHPLC–MS/MS

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Cyanobacteria or blue-green algae are common throughout the world. Microcystins are cyclic heptapeptides produced by cyanobacteria and associated with human hepatotoxicity. Under certain nutrient rich and environmentally favourable conditions, freshwater cyanobacteria have the potential to rapidly accumulate and form blooms. Concentrations of microcystins in water containing blooms can be highly variable and must be known for safe water supplies. Due to the hepatotoxic nature, the World Health Organisation (WHO) has set a provisional limit of 1 microgram / L for total Microcystin-LR as a marker for cyanobacteria toxin levels. Initial spiked water samples allowed optimisation of chromatography for separation and MS conditions for low level detection. This approach offers reproducible analyte recoveries and rapid sample preparation times. The Bruker EVOQ Elite triple quadrupole detector was employed to monitor appropriate MRM transitions and analyte separation was provided by an ACE Excel 2 micron C18 column. Experiments included optimisation of the MS conditions and transitions for sensitivity and reproducibility across the concentration ranges explored. A rapid UHPLC method providing satisfactory separation and elution of Microcystin-LR (MC-LR), Microcystin-RR (MC-RR) and Microcystin-YR (MC-YR) in under 3.5 minutes was developed using the Bruker Advance UHPLC with an ACE Excel 2 micron C18 UHPLC column. Signal response studies indicated an improvement in sensitivity of x128 fold and x194 fold for MC-LR and MC-YR respectively when detecting the transition products of the doubly charged ions which dominate the mass spectra under full scan conditions. All three mycrocystins share the common m/z 135 product ion. The method was found to be linear for MC-LR, MC-RR and MC-YR from 0.05ppb to 50ppb with regression coefficient values (R^2) = 0.999, 0.999 and 0.997 respectively. Repeatability at the 0.05ppb concentration level for MC-LR, MC-RR and MC-YR was demonstrated with RSD values (n = 7) of 10%, 7.1% and 8.1% respectively.

Keywords: *cyanobacteria, microcystin, water, UHPLC–MS/MS, trace analysis*

F43
OCCURENCE OF ORGANIC CONTAMINANTS IN FOOD SUPPLEMENTS BASED ON FISH OIL

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In the last decade, a consumption of fish oil supplements has increased worldwide. They are recommended as an additional diet source of omega-3, omega-6 fatty acids, minerals and vitamins. However, fish oil may contain relatively high concentrations of organic pollutants, especially halogenated, such as polychlorinated biphenyls (PCBs), various organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and also polycyclic aromatic hydrocarbons (PAHs). Therefore, the consumption of nutritional supplements with oil components may increase a dietary intake of various POPs and PAHs. During the recent years several RASFF notifications on PAHs, in fish oil supplements were released. The maximum limit set by the Regulation No. 1881/2006/EC on PAH 4 (sum of benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, and chrysene) for oils and fats intended for direct human consumption is 10 $\mu g.kg^{-1}$. The main aim of this study was to analyse altogether 44 dietary supplement products based on fish oil available at the Czech market and to assess levels of halogenated POPs and PAHs. The rapid and simple sample preparation procedure for the simultaneous isolation of the above mentioned target contaminants from fish oil supplements is based on a extraction/clean-up step employing solid-phase extraction (SPE) on silicagel minicolumn followed by two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOFMS) was used. Needed to emphasize that this procedure enables simultaneous determination of PAHs, PCBs, PBDE and OCPs, thus in this way significant increase of laboratory throughput is achieved. The levels of PAHs and POPs in analyzed samples were 0.35-53.7 $\mu g.kg^{-1}$ for Σ8PAHs, 0.20-55.7 $\mu g.kg^{-1}$ for Σ7PCBs and 0.2–10.3 $\mu g.kg^{-1}$ for ΣPBDEs. Three of the analyzed samples exceed the maximum limit set by the Reg. 1881/2006/EC for PAH 4.

Keywords: *food supplements, GCxGC TOFMS, POPs, PAHs, solid-phase extraction*

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F44
„LOOSE THE LIPIDS, FIND YOUR ANALYTES“ – THE IMPLEMENTATION OF NEW SORBENT FOR D-SPE IN ANALYSIS OF ORGANOHALOGEN COMPOUNDS IN FOOD AND COMPLEX BIOLOGICAL MATRICES

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Interference from lipids is a typical problem in trace analysis of food contaminants in fatty or complex biological matrices. Lipids can build up in the instrument, which results in a lower lifetime of a chromatographic column and reduction of analytes' sensitivity due to ion suppression. Therefore there is a lot of emphasis on quick and effective clean-up techniques, such as d-SPE, which are often used within a very frequently performed QuEChERS method. EMR – Lipids (Enhanced Matrix Removal)[1] is a new sorbent that selectively removes lipids from extracts of complex matrices and challenging high-fat samples.
A main focus of this study was to evaluate purification potential of EMR for fatty foods such as fish. This matrix also belongs to a routinely monitored material for assessing environmental contamination by halogenated contaminants. This contribution is focused on the simultaneous determination of 10 brominated flame retardants (BFRs) and 19 per- and polyfluoroalkylated compounds (PFASs) using ultra-high performance liquid chromatography coupled with tandem mass spectrometry (U-HPLC–MS/MS).
A smoked trout sample (fat content 10%) was extracted by the QuEChERS method using acetonitrile as extracting solvent followed by the purification step with dispersive solid phase extraction (d-SPE). For purification EMR and other commonly used sorbents, such as C18 and Z-Sep, were tested in order to evaluate lipid clean-up efficiency, as well as their effect on analytes' recovery.
The QuEChERS extraction method with a clean-up step using the new sorbent EMR has been successfully validated with recovery 70–120%, RSD 23% and LOQ 0.002–0.02 ng/g for PFASs and 0.05–0.1 ng/g for BFRs. The newly validated method was subsequently applied for the extraction of fatty fish (fat content 10%) and seafood samples.

[1] Collective of Authors: PAH Analysis in Salmon with Enhanced Matrix Removal. Agilent Technologies [Online] 2015. https://www.agilent.com/cs/library/applications/5991-6088EN.pdf

Keywords: *EMR, Fatty matrices, d-SPE, Organohalogen compounds, U-HPLC–MS/MS*

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F45
THE ANALYSIS OF CHLORINATED DIOXINS AND DIFURANS IN PET FOOD

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Introduction: Screening for chlorinated dioxins and difurans in human supplies is well-established. Both the EU and US have extensive protocols for testing of food for human consumption and additives to feed for commercial livestock. Given recent contamination issues in pet food (e.g. 2007 melamine incident), there is a growing concern for safety. With increasing efforts to screen finished pet foods and additives, development of reliable analytical techniques is a high priority. With high lipid content in canned and dried pet foods, Persistent Organic Pollutants (POPs) are likely to be found in them. Use of automated extraction and clean up of pet food samples can deliver extracts for same day analysis. Procedures for processing canned dog food for PCDD/Fs (EPA1613) are described. Method: Three brands canned dog food were analyzed. 10g of sample was spiked with ^{13}C labeled standards and mixed with diatomaceous earth for drying. Samples were put in extraction cells topped with Ottawa sand. Pressurized Liquid Extraction with 50/50 dichloromethane/hexane (120°C, 1500psi, 20 min) was carried out followed by volume reduction and solvent exchange to hexane. Cleanup was done using automated column chromatography (acid-base-neutral silica, alumina, carbon columns). Samples were loaded across the ABN silica columns and eluted onto the alumina columns. Then the samples were eluted onto the carbon column using dichloromethane. The eluate was collected (fraction # 1). The carbon column was eluted with toluene collected (fraction # 2). Fractions were reduced in volume and analyzed with high resolution GC/MS. Preliminary Data: Canned dog food was extracted and cleaned up with the techniques described and analyzed for PCDD/Fs. Recoveries for PCDD/F (averages): tetra-CDD/F 88%; penta-CDD/F 74%; hexa-CDD/F 93%; hepta-CDD/F 81%; octa-CDD/F 73%. Detection limits for all native species measured were < 0.1 pg/g dog food. Review of the 3 pet food matrices showed labeled recoveries well within EPA1613 limits. Analysis of the method blank showed no background levels above the CS 0.1 calibration standard level except for OCDD. Combining the clean background with good recoveries demonstrates the ability of Pressurized Liquid Extraction and automated column chromatography to handle wet pet food of various types. With a total processing time of ~5 hours from start to finish, this enables sample turnaround for same day analysis. When factoring in the need to qualify batches for release and product delays, the value of rapid testing become critical. Novel aspect: Automated extraction and clean up of pet food samples and additives followed by same day POPs analysis is now possible.

Keywords: *pet food analyis, dioxins and furans, PCBs, automated sample prep, GC/MS*

F46
THE ANALYSIS OF CHLORINATED DIOXINS, DIFURANS AND POLYCHLORINATED BIPHENYLS IN EDIBLE OILS

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Introduction: Polychlorinated dibenzo-p-dioxins and furans are a group of 210 compounds, of which 17 are considered toxic. These have a 2,3,7,8-chlorine substitution pattern. They are of great concern to human health. Twelve polychlorinated biphenyls (out of a total of 209 compounds) have also been identified as human toxins. All these analytes bio accumulate in adipose tissue and end up in food supplies. For this reason, the FDA and EU have established strict regulations for monitoring of food products for human consumption, in particular edible oils. Manual extraction of oils is a time consuming procedure delaying lab turnaround times. By automation, food oil samples can be reliably processed within 24 hours. The following procedure utilizes an automated sample clean-up system.
Method: 5 g of various oil matrices (lard, olive oil, corn oil, cod oil, red palm oil, unrefined pumpkin oil, and unrefined vegetable oil) were spiked with ^{13}C labeled standards, diluted into hexane and drawn up in a gas-tight syringe. Columns (acid-base-neutral silica, alumina and carbon) of the automated chromatography clean up system were conditioned. Samples were then loaded across the ABN silica columns and eluted onto the alumina columns. Then the samples were eluted onto the carbon column using dichloromethane. The eluate was collected as fraction # 1. The carbon column was eluted with toluene collected as fraction # 2. The fractions were reduced in volume and analyzed with high resolution GC/MS.
Preliminary Data: Edible oils were cleaned up with the technique described and analyzed for PCBs and PCDD/Fs. Recoveries for PCBs (averages) were: tetra-CBs 69%; penta-CBs 76%; hexa-CBs 64%; hepta-CBs 79%. Recoveries for PCDD/F (averages): tetra-CDD/F 74%; penta-CDD/F 82%; hexa-CDD/F 71%; hepta-CDD/F 78%; octa-CDD/F 70%. Detection limits for all native species measured were < 0.5 pg/g oil. Analysis of the 6 matrices processed yielded good recoveries for all analytes. Analysis of an *n*-hexane blank sample resulted in no detectable target analytes measured within the calibration range of each respective compound. With a total processing time of less than 2.5 hours, the automated column chromatography system and concentrator deliver an efficient, completely automated sample prep process for edible oils.
Novel aspect: Quick analysis of POPs (Persistent Organic Pollutants) in edible oils is now possible using automated column chromatography yielding good recoveries.

Keywords: *dioxins, furans, PCBs, automated sample prep, GC/MS*

F47
AUTOMATED PRESSURIZED LIQUID EXTRACTION AND CLEAN UP OF SEA FOOD SAMPLES IN THE ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS (POPS)

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Introduction: The occurrence of polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs) and biphenyls (PCBs) in a variety of sea foods has been amply documented. This includes fish for human consumption and crab meat from around the world, including in crabs from the St Louis Bay and China. Some evidence has been found for a relationship between concentrations in sediment and fish tissue. The seventeen laterally chlorinated dioxins and furans are generally considered the most toxic, as are the so-called dioxin-like PCBs. Analyses of sea food samples using US EPA methods 1613 (PCDD/Fs) and 1668 (PCBs) have been carried out worldwide. Traditional Soxhlet extraction and sample cleanup are time consuming and can result in data of low quality and reproducibility. As an alternative to obtain faster and more reliable data, these various steps have been automated. Method: Between 5 and 10 g of sample was spiked with ^{13}C labeled PCDD/F and PCB standards and mixed with Hydromatrix™. Samples were put in extraction cells. Pressurized Liquid Extraction with 50/50 *v/v* dichloromethane/hexane (120°C, 1500 psi, 20 min) was carried out followed by volume reduction and solvent exchange to hexane. Cleanup was done using automated column chromatography (jumbo acid silica, acid-base-neutral silica, alumina, carbon/celite columns). Samples were loaded across the silica columns and eluted onto the alumina column using hexane. The alumina was eluted with 2/98 *v/v* and 50/50 *v/v* dichloromethane/hexane, collecting PCBs as fraction 1. Elution of PCDD/Fs and co-planary PCBs onto the carbon/celite column was followed by their collection in toluene (fraction 2). Fractions were reduced in volume and analyzed with high resolution GC/MS.
Data: Crab meat showed low concentrations of PCDD/F, between 0.05 and 0.35 pg/g. Recoveries of the ^{13}C isotope dilution standards were excellent varying from 87% to 95%. A certified reference sample of fish with known composition was analyzed. Excellent agreement was found between native values of PCDD/F measured (20–170 pg/g) and the reference values provided. ^{13}C recoveries ranged from 83% to 93%. For the PCBs in the fish native values varied from 2 pg/g to 612 pg/g, also in excellent agreement with the certified concentrations. ^{13}C recoveries were between 50–90%.
Novel aspect: Automated extraction and clean up of sea food samples followed by same day POPs analysis is now possible.

Keywords: *sea food contaminants, POPs, Automated Sample Prep, GC/MS*

F48
INFANT FORMULA AND MILK: CHLORINATED DIOXINS AND FURANS ANALYSIS USING AUTOMATED EXTRACTION AND COLUMN CHROMATOGRAPHY

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Infant Formula and Milk: Chlorinated Dioxins and Furans Analysis Using Automated Extraction and Column Chromatography Introduction: Because of important public health considerations for children, infant formula has been tested for Persistent Organic Pollutants (POPs) such as polychlorinated dibenzo-p-dioxins (PCDDs) and furans (PCDFs). Typically concentrations of these analytes found are lower than in human breast milk but continuous monitoring is important. This requires well equipped laboratories capable of trace level analysis work, as often PCDD/Fs amounts are at the picogram or femtogram level. We also included samples of freeze-dried and regular cow milk. Analyses of infant formula and milk samples using US EPA methods 1613 (PCDD/F) have been carried out worldwide. Traditional Soxhlet extraction and sample cleanup are time consuming and can result in data of low quality and reproducibility. As an alternative to obtain faster and more reliable data, these various steps have been automated. Method: Between 1 and 8 g of sample was spiked with ^{13}C labeled PCDD/F standards and mixed with Hydromatrix™. Samples were put in extraction cells. Pressurized Liquid Extraction with 50/50 *v/v* dichloromethane/hexane (120°C, 1500 psi, 20 min) was carried out followed by volume reduction and solvent exchange to hexane. Cleanup was done using automated column chromatography (jumbo acid silica, acid-base-neutral silica, alumina, carbon/celite columns). Samples were loaded across the silica columns and eluted onto the alumina column using hexane. The alumina was eluted with 2/98 *v/v* and 50/50 *v/v* dichloromethane/hexane to remove PCBs (waste). Elution of PCDD/Fs onto the carbon/celite column was followed by their collection in toluene (Fraction 1). Fractions were reduced in volume and analyzed with high resolution GC/MS.
Data: Infant formula showed low concentrations of PCDD/F, between 0.20 and 76 pg/g for the various congeners, OCDD being the highest. Recoveries of the ^{13}C isotope dilution standards were excellent varying from 80% to 100%. PCDD/F concentrations found in both kinds of milk were low with highest concentrations around 1 pg/g. This low level of detection demonstrated the sensitivity of the method used. Freeze-dried milk gave better ^{13}C labeled recoveries (75–100%) than regular milk (45–80%) because of the absence of water.
Novel aspect: Automated extraction and clean-up of infant formula and milk samples followed by same day POPs analysis is now possible.

Keywords: *automated sample prep, Dioxins and furans, Infant formula and milk, POPs*

F49
AUTOMATED PRESSURIZED LIQUID EXTRACTION AND CLEAN UP OF EGG YOLK AND PEANUT BUTTER SAMPLES IN THE ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS (POPS)

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Introduction: Polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs) and biphenyls (PCBs) are lipophilic nature; these analytes bioaccumulate in adipose tissue and end up in food supplies, such as fish, meats, oils, and poultry. For this reason, the U.S. FDA and EU have established strict regulations for the monitoring of food products for human consumption. These compounds have been found in eggs and it is estimated that about 4% of daily PCDD/F intake in human diet is because of egg consumption. Because of its lipid content the PCDD/Fs are mostly concentrated in the egg yolk. Peanut butter can contain up to 50% fat. Analyses of egg yolk and peanut butter samples using US EPA methods 1613 (PCDD/Fs) and 1668 (PCBs) have been carried out worldwide. Traditional Soxhlet extraction and sample cleanup are time consuming and can result in data of low quality and reproducibility. As an alternative to obtain faster and more reliable data, these various steps have been automated.
Method: 5 g of peanut butter or 17 g of egg yolk was spiked with ^{13}C labeled PCDD/F and PCB standards and mixed with Hydromatrix™. Samples were put in extraction cells. Pressurized Liquid Extraction with 50/50 *v/v* dichloromethane/hexane (120°C, 1500 psi, 20 min) was carried out followed by volume reduction and solvent exchange to hexane. Cleanup was done using automated column chromatography (jumbo acid silica, acid-base-neutral silica, alumina, carbon/celite columns). Samples were loaded across the silica columns and eluted onto the alumina column using hexane. The alumina was eluted with 2/98 *v/v* and 50/50 *v/v* dichloromethane/hexane, collecting PCBs as Fraction 1. Elution of PCDD/Fs and co-planary PCBs onto the carbon/celite column was followed by their collection in toluene (Fraction 2). Fractions were reduced in volume and analyzed with high resolution GC/MS.
Data: Egg yolk showed low concentrations of PCDD/F at a total of ~ 1 pg/g, mostly OCDD. Recoveries of the ^{13}C isotope dilution standards were excellent varying from 70% to 95%. Peanut butter contained PCDD/F concentrations below detection limits and also had ^{13}C isotope dilution standards recoveries varying from 70% to 95%. Native PCBs concentrations were up to 7 pg/g for the various congeners with ^{13}C recoveries between 60–75%.
Novel aspect: Automated extraction and clean-up of food samples such as egg yolk or peanut butter followed by same day POPs analysis is now possible.

Keywords: *eggs and peanut butter, POPs, automated sample prep, GC/MS*

F50
CHLORINATED DIOXINS, FURANS AND BIPHENYLS ANALYSIS IN BEVERAGES USING AUTOMATED EXTRACTION AND REDUCED SOLVENT VOLUME COLUMN CHROMATOGRAPHY

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Introduction: Persistent organic pollutants (POPs) such as polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) and biphenyls (PCBs) have been a major environmental concern for a number of decades. Their presence in various foods has also been of concern. The US FDA has carried out monitoring of various food stuffs for PCDD/Fs and PCBs. This includes analysis of beverages for these toxic compounds. We analyzed both coffee beans and cranberry pulp. Routine analysis of these compounds follows US EPA method 1613 (PCDD/Fs) and 1668 (PCBs). Traditionally sample processing has involved multi-day Soxhlet extraction and manual sample clean up using column chromatography. As an alternative to obtain faster and more reliable data, these various steps have been automated.
Method: 10 g of sample was mixed with Hydromatrix™. Samples were put in extraction cells. Pressurized Liquid Extraction with 50/50 *v/v* dichloromethane/hexane (120°C, 1500 psi, 20 min) was carried out followed by volume reduction and solvent exchange to hexane. At this point samples were spiked with ^{13}C labeled standards for quantification. Cleanup was done with automated column chromatography, using high capacity acid-base-neutral silica, alumina and carbon/celite columns (for cranberry pulp) or high capacity acid-base-neutral silica and alumina columns (coffee beans). With cranberry pulp solvents used were: hexane (to bring analytes on alumina), 10/90 dichloromethane/hexane (to remove PCBs to waste), dichloromethane (to transfer PCDD/Fs onto carbon/celite column) and toluene (to elute PCDD/Fs off carbon collected as Fraction 1). With coffee beans hexane (to bring analytes onto alumina) and 10/90 dichloromethane/hexane (to collect PCBs as Fraction 1) were used. Fractio ns were then reduced in volume and analyzed with high resolution GC/MS. Data: Cranberry pulp gave ^{13}C PCDD/F recoveries of 60–90%. Coffee beans gave ^{13}C PCBs recoveries between 70–85%. Since samples were spiked after extraction and before clean up these numbers represent the labeled recoveries across the cleanup step.
Novel aspect: Automated extraction and clean-up of beverages samples followed by same day POPs analysis is now possible Our new reduced volume column chromatography program uses less than a total of 400 mLs of solvents with a maximum processing time of 40 mins.

Keywords: *beverages analyses, persistent organic pollutants, automated extraction and clean up, GC/MS*

F51
CRITICAL ASSESSMENT OF CLEAN-UP TECHNIQUES EMPLOYED IN ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS AND PERSISTENT ORGANIC POLLUTANTS IN FATTY SAMPLES

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Purification is a very important and in many cases critical step in analysis of polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs). Removing of matrix co-extracts from primary extracts improving results in selectivity and sensitivity of trace analysis.
In this study Enhanced Matrix Removal—Lipid (EMR – Lipids), a new sorbent for selective removing of lipids in complex biotic matrices, was tested for the determination of 16 carcinogenic polycyclic aromatic hydrocarbons (PAHs), 17 polychlorinated biphenyls (PCBs), 22 polybrominated diphenyl ethers (PBDEs) and 23 organochlorine pesticides (OCPs) in fish and seafood. For the extraction of the smoked trout sample (fat content 15 % *w/w*) QuEChERS method followed by purification of acetonitrile phase using dispersive solid phase extraction (d-SPE) was used. For the comparison of the clean-up efficiency and also influence on the analyte recoveries, the same sample was also processed by routinely used ethylacetate based extraction method followed by clean-up using SPE on silica column. For the determination of target PAHs and POPs in purified extract gas chromatography coupled with tandem mass spectrometry (GC–MS/MS) was used.
The results show that the new clean-up strategy is applicable for the determination of PAHs and POPs in high fatty matrices such as fish. Employing rapid inspection of purified extracts by direct analysis in real time coupled with time-of-flight-mass spectrometry (DART–TOFMS) – fingerprinting significant decrease of triacylglycerols (TAGs) content in samples cleaned using tested d-SPE was achieved. The performance characteristics obtained by the newly implemented clean-up procedure were as follows: recoveries 70-120 % and RSD < 20 % at 2 $\mu g.kg^{-1}$ for PBDEs, PAHs and 5 $\mu g.kg^{-1}$ for OCPs, PCBs spiking level. This fast method enabling high laboratory throughput was used for the determination of PAHs and POPs in fatty fish and seafood.

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F52
EPA METHOD 557 QUANTITATION OF HALOACETIC ACIDS, BROMATE AND DALAPON IN DRINKING WATER USING ION CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

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Haloacetic Acids (HAAs) can be formed during drinking water purification in municipal water supplies during the chlorination, ozonation or chloramination of water. Reactions between chlorine and organic matter present in the water can create HAAs.There are health concerns regarding human consumption of HAAs.Because of these concerns, the US EPA published Method 557 for the quantitation of HAAs using Ion Chromatography coupled to Tandem Mass Spectrometry (IC–MS/MS). Previous techniques for the analysis of HAAs included derivitization of the HAAs and analysis by GC–MS. The IC–MS/MS method bypasses derivitization of the HAAs and allows direct analysis of the drinking water samples without time consuming sample preparation, aside from internal standard addition and preservatives during sample collection. Drinking water samples were collected and treated with the preservatives. Calibration standards were prepared by spiking drinking water with 9 Haloacetic Acids, bromate, and dalapon stock solutions. The standards concentration ranged from 0.25 to 20 µg/L. 100 µL of the spiked water and blank samples were injected directly onto the IC column (Thermo Scientific™ Dionex™ IonPac™ AS-24 250×2). The compounds are separated using a gradient elution of KOH (Thermo Scientific Dionex ICS-5000 HPIC) and analyzed via mass spectrometery (Thermo TSQ Endura). Prior to the IC eluent entering the MS it passes through an electrolytic suppressor to remove potassium via ion exchange. The following HAAs were analyzed, along with bromate and the pesticide dalapon: Bromochloroacetic acid (BCAA), Bromodichloroacetic acid (BDCAA), Chlorodibromoacetic acid (CDBAA), Dibromoacetic acid (DBAA), Dichloroacetic acid (DCAA), Monobromoacetic acid (MBAA), Monochloroacetic acid (MCAA), Tribromoacetic acid (TBAA) and Trichloroacetic acid (TCAA). While US regulations currently only require the monitoring of 5 of the HAAs, (MCAA, DCAA, TCAA, MBAA and DBAA), interest is growing i n the additional 4 HAAs and were included in this analysis. Drinking water samples were tested using municipal drinking water as well as bottled water for a variety of manufacturers to test for the presence of HAAs. The response of the HAAs, bromate, and dalapon over the concentration range was linear. An instrument detection limit (IDL) was calculated for each analyte based on replicate injections and the student's t-test. Of utmost importance to the ICMS/MS analysis is the removal of potassium from the IC eluent. The electrolytically regenerated suppressor accomplishes this via an ion exchange membrane, exchanging H^+ ions for K^+ ions as the eluent flows through the suppressor. The eluent leaving the suppressor is now compatible with ESI sources. A divert valve installed prior to the electrospray source was utilized to divert Cl^-, Carbonate, SO_4^- and NO_3^- from the source. A mixingT was installed between the IC column and the ESI source to add isopropyl alcohol to the water to improve ionization.

Keywords: *environmental, ICMS, haloacetic acid, triple quadrupole, drinking water*

FOODOMICS
(G1 – G11)

G1
ULTRASTRUCTURAL AND CHEMICAL CHANGES INDUCED BY THYMUS VULGARIS ESSENTIAL OIL ON STAPHYLOCOCCUS AUREUS AND BACILLUS CEREUS CELL WALL

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Abstract Aims: To investigate the action of Thymus vulgaris essential oil on Staphylococcus aureus and Bacillus cereus cell wall. Methods and Results: The effect of thyme essential oil on S. aureus and B. cereus was evaluated by bio-assay preparation and minimal inhibitory concentrations (MICs). Ultrastructural changes were assessed using gram staining, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Exposure to thyme oil induced alterations in the bacterial membrane of S. aureus and B. cereus, which led to loss of cell wall integrity, as demonstrated by gram staining, SEM and TEM. In addition, loss of cellular contents, irregular cytoplasmic membrane, swollen cells; shrinkage of the cell, incomplete cell division and the presence coagulated material, as indicated by SEM and TEM were observed. Chemical changes where investigated by assessing changes in fatty acid composition of S. aureus and B. cereus cells exposed to thyme essential oil. Treatment with thyme oil depleted both saturated and unsaturated fatty acids of S. aureus and B. cereus. In addition, unsaturated C18:1n9 (cis), 18:3n6 and C18:2n6 (cis) fatty acids were completely depleted when using the undiluted and diluted thyme oil in comparison to control showing that the more concentrated the oil is, the more degradation of fatty acids occurs. Protein changes where investigated using mass spectrometry based proteomics for S. aureus and B. cereus cells exposed to thyme oil. The results showed differences in protein abundance between treated and untreated samples. Conclusions: Thyme oil unlike antibiotics, targets bacterial different components of the bacterial cells at the same time which eventually results in cell death. Significance and Impact of the Study: Thyme essential oil shows effective antimicrobial activity. Evidence provided in this study indicates that thyme essential oil might enhance the chances of developing new conventional and natural antimicrobial agents (drugs as well as food preservatives) and be good alternatives to synthetic chemicals.

Keywords: *antibacterial activity, thymus vulgaris essential oil, morphological changes, fatty acid profile, proteomics.*

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G2
IN VITRO DIGESTION OF PARMIGIANO REGGIANO CHEESE: CHARACTERIZATION OF THE NITROGEN FRACTION

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Parmigiano Reggiano is a hard long ripened cheese produced in a restricted Italian area and bearing a Protected Designation of Origin. It is produced from bovine milk, added of rennet and natural whey starter. The curd is heated at 55°C, and the product is ripened at least for 12 months, even if much longer ageing time are usually adopted. In this period, the residual rennet activity, and the proteolytic enzymes of the Lactic Acid Bacteria (starter and non starter), contribute to casein breakdown into shorter peptides, with a wide range of molecular weight. Caseins are initially hydrolyzed by curd and milk proteolytic enzymes to large- and intermediate-size peptides, which, in turn, are hydrolyzed by LAB and NSLAB proteinases and peptidases to short peptides and aminoacids (Mc Sweeney, 2004). This proteolytic process induces a better digestibility of cheese compared to intact milk, and leads also to a decrease in its allergenicity.

Peptide composition of water soluble extract of cheese has already been fully characterized, demonstrating several trends in the composition according to the ageing time (Sforza et al., 2012). Moreover, the accumulation of non proteolytic aminoacyl derivatives was demonstrated during ripening.

Given the relevant physiological influence of cheese proteolysis on human health (digestibility, bioactive peptides, allergenicity, and others), in this work Parmigiano Reggiano cheese at different ripening times (16, 24 and 36 months) was submitted to simulated gastrointestinal digestion, according to the recently approved method of Minekus et al (2014). The peptidic profile generated after in vitro digestion was compared with the peptidic profile of the water soluble extract. In this way it was possible to identify the compounds that were resistant to digestion, so that potentially can be absorbed and exert their physiological activity. Moreover, new peptides are generated from casein digestion. Beside the nutritional point of view, the new generated peptides can contain sequences with bioactive activity. The use of tandem mass spectrometry allowed to identify the nitrogen compound generated, showing, as expected, a decrease or complete disappearing of high molecular weight peptides (MW>2000 Da) and the presence of many lower molecular weight peptides (MW<1000 Da). Non proteolytic amino acyl derivatives were found not to be resistant to simulated *in vitro* digestion.

The characterization of the peptides generated after digestion is of outmost importance to understand the potential biofunctional activity of Parmigiano Reggiano.

Keywords: *cheese, peptides, gastrointestinal digestion, mass spectrometry, non proteolytic aminoacyl derivatives*

G3
PEPTIDOMIC TOOLS FOR PROTEOLYSIS CHARACTERISATION IN DRY-CURED HAM

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There is an increasing interest in the study of naturally generated peptides because of their numerous applications in foods as processing biomarkers and/or bioactive compounds. However, due to the non-specificity in their generation, the use of typical mass spectrometry strategies for their identification is not possible. Main difficulties in the study of naturally generated peptides arises from both, the small size of these fragments that cannot be trypsin digested and sometimes are in the limit of some mass spectrometry techniques, and the impossibility of controlling the hydrolysis, obtaining a complex mixture of peptides from different proteins with unspecific cleavage sites. For this reason, the use of peptidomic approaches based on mass spectrometry in tandem is essential to elucidate the sequence of such naturally generated small peptides. Proteolysis during dry-cured ham processing is the main responsible for the generation of large amounts of free amino acids as well as thousands of small peptides as intermediate proteolysis products which influence the characteristic texture, aroma and flavor of the final product. The intensity of the proteolysis is highly dependent on the activity of the endogenous endo- and exo-peptidases which is strongly affected by processing conditions such as the amount of salt, temperature and humidity of the curing room, time of ripening, etc. For this reason, the long ripening processing, characteristic of high-value dry-cured hams such as Iberian ham, results in a stronger proteolysis in comparison with standard ripened dry-cured hams. In this work, peptidomic tools have been applied to both types of products, 2-years ripened Iberian ham vs 1-year ripened ham, focusing on small peptides within the range 6 to 20 residues. The main results show relevant differences between both typ es of hams, not only in the proteolysis chain but also in the number of released peptides which is larger for the long ripened hams. This confirms the more intense proteolysis phenomena in longer processes. Thus, peptidomics constitute an indispensable tool to develop fast and precise strategies for the identification of released peptides in complex biological samples like dry-cured products.

Keywords: *peptidomics, proteomics, peptides, proteolysis, dry ham*

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G4
HILIC–UHPLC–ESI(+)-HRMS BASED METABOLOMICS SCREENING MODEL TO DETECT ANABOLIC ABUSE IN BOVINE ANIMALS

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The use of anabolic agents in meat producing animals is banned within the EU but illegal administrations are reported to continue. Present testing systems to detect drug abuse are compromised by the use of endogenous hormones, designer drugs and low-dose chemical cocktails but also by the limited number of samples which can be analysed routinely for drug residue presence. In this context, metabolomics-based analysis focusing on the identification of metabolite profiles representative of biological responses to exogenously administered agents offers potential as a new screening strategy. The aim of the present study was to determine if untargeted high resolution mass spectrometry-based metabolomics of urine could be used as a predictive tool for identification of anabolic steroid abuse in cattle. Urine samples (collected from calves (n=6) pre- and post-boldenone undecylenate administration) following a minimal sample preparation procedure were analysed using HILIC–UHPLC– (ESI+)–HRMS. Before multivariate statistical analysis, MS data pre-treatment including scaling and/or log-transformation were applied to all variables (ions) to enhance data quality and to make classification and clustering more effective. Statistical analysis of acquired urinary metabolomics fingerprints facilitated discrimination between samples collected pre- and post-steroid administration, and the selection of ions contributing most significantly to discrimination between animal treatment groups. Focus on these ions of interest led to elimination of artefacts and enabled selection of candidate biomarkers of boldenone undecylenate administration. The fitness of the OPLS–DA model based on these selected features (ions) was R2(Y)=0.883 and Q2=0.833 after performing 7-fold cross validation, permutation test - R2=(0.0, 0.139) and Q2=(0.0, -0.366) for 999 permutations, and CV-ANOVA (p=5.14413×10-31) confirmed the robustness of the established model. The results indicate that the new model presents good descriptive and predictive capabilities enabling use as a predictive tool for the classification of urine samples of unknown status which could serve as a new and alternative screening strategy to detect illegal anabolic practices in cattle. To date 155 bovine urine samples of known and unknown treatment status originating from animals of varying breed, gender, age, geographical region and diet were randomly analysed using the established model to assess the relevance and robustness of the model upon variable factors. All samples coming from known untreated animals were classified without misallocation. In the case of samples coming from animals of unknown treatment status, the majority of samples were classified as "untreated" with the exception of four suspect samples classified as "treated". Ongoing work is focused on the identification and biological interpretation of selected urinary metabolite markers utilised within co nstructed models.

Keywords: *LC-HRMS metabolomics, anabolic practices, urine, screening, food safety*

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G5
A TWO-YEAR STUDY ON NMR-BASED METABOLIC PROFILING OF APPLE STORED UNDER DESIGNED-CO2 CONDITION OR TREATED WITH 1-MCP

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Our project has pursued an NMR-based metabolic profiling study on apple since 2013. In order to verify the repeatability of NMR-based metabolic profiles and make an intensive study on apple's characters, we carried out a two-year comparative study. Two cultivars of apples harvested in picking season of 2013 and 2014 in Aomori prefecture in Japan were stored under different conditions: (i) Orin treated with 1-methylcyclopropene (1-MCP) and (ii) Fuji under high or low CO_2 concentration. The metabolic variation of apples was characterized at storage time of 0, 4, and 6 months respectively. The metabolic variations of principal carbohydrates in apple are consistent with in both years. Along with the storage duration the amount of Glc increased, whereas that of Suc decreased, and the Fru tended to increase. This variation can be explained by 'futile recycles', in other words: the sugar metabolism in apple was progressing in secret during storage [1]. Princi pal component analysis (PCA) score plots showed a tendency of classifying the samples with-without 1-MCP treatment after 4-months storage, and the trend became stronger with the increase of the storage period. After excluding the intense signals of carbohydrates and the data of 0 month storage, using OPLS–DA model the samples are separated into two classes clearly. The VIP plot demonstrates that EtOH, MeOH, malic acid (MA), Ala and overlapped signal assigned to citramalic acid (MeMA) and lactic acid (LA) are the top 5 variables being responsible for distinguishing MCP treated samples from non-treated ones. Significant changes in these compounds were observed between the samples treated with or without 1-MCP. For samples without 1-MCP treatment, as the time elapses EtOH, MeOH and MeMA increased and MA decreased. Meanwhile, in samples treated with 1-MCP, all these compounds were kept at low level during the storage. During four months, no classification can be seen between the Fuji apples stored under high and low CO_2 conditions. Based on the three main metabolites: carbohydrates, organic acid, and amino acid, samples in both years showed almost the same metabolic patterns. Under low CO_2 condition, Glc was increased with the lapse of storage time. The increase of Glc also occurred more obviously under high CO_2 condition. Moreover, the PCA score plot with all variables showed a clear separation between the two-year harvested apples for both Fuji and Orin. Differential abundance of MA, EtOH and sorbitol may contribute to this separation. MA in the Orin is rich in 2013, whereas sorbitol and EtOH are rich in 2014. Considering the difference in the biosynthesis of sorbitol and EtOH, the oxygen level might plays a role for the enrichment of both compounds [1, 2]. The results in current study indicate that NMR-based metabolic approach is reliable and supportable to make comprehensive on the metabolomics study for fruit.

[1] PLoS ONE 7 (2012) e33055. [2] Postharvest Biol Technol 62 (2011) 295-304.

Keywords: *NMR, metabolic profiling, apple fruit, 1-MCP treatment, designed-CO_2 condition*

Acknowledgement: *The project of Ministry of Agriculture, Forestry and Fisheries, Japan, "Development of non-destructive technology evaluating various qualities of agricultural products"*

G6
TARGETED AND UNTARGETED LC–MS METABOLIC PROFILING FOR PROVING THE "FRESHLY-FROZEN" CLAIM IN FISH

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The aim of this study is the application targeted and untargeted LC–MS methods in order to substantiate the "freshly-frozen" claim in fish. The claim "freshly-frozen" is a combination of food safety of frozen branded fish and at the same time the good appearance, flavour and odour of fresh non-branded fish. For this purpose, the differences, caused in metabolic profile of fish during the first hours after fishing, were studied, using two approaches: targeted determinations and untargeted LC–qToFMS profiling. The study was focused on Sparus aurata (sea bream) and the samples were from aquacultures in Porto-Buffalo, Evia, Greece. The chosen time points were 0, 6, 12, 24, 36, 48 h after fishing. Some random samples were analysed as it happens in standard fishing and freezing procedures. The most important freshness indices were determined in targeted approaches: total volatile basic nitrogen (TVBN), biogenic amines and nucleotides profile, which include d the products of ATP degradation (ATP, ADP, AMP, IMP, INO, Hx), were determined. In untargeted approach, the same samples were analysed with LC-qToFMS in positive and negative ionization with RP–LC and HILIC separation modes. The data was analyzed with Partial Least Square-Discriminant Analysis (PLS–DA), in order to detect if there is some differentiation of the metabolic profile between the time points. The results of targeted approach show that nucleotides degradation is the most appropriate index for short-term changes and the critical point is 36-hour after fishing in order to be a significant differentiation. In the untargeted approach, only positive RP–LC and HILIC separation modes show a significant change in metabolic profile. From the results of PLD–DA, there is an agreement with targeted approach that 36 hours are a crucial point for the change in metabolic profile.

Keywords: *freshly-frozen, fish, targeted determination, untargeted screening*

Acknowledgement: *We would like to acknowledge financial support by Kallimanis S.A. (http://www.kallimanis.gr/en)*

G7
NMR-BASED METABOLIC PROFILING OF 1-METHYLCYCLOPROPENE (1-MCP)–TREATED JAPANESE APPLES

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The apple is a climacteric fruit; these are characterised by climacteric increases in ethylene production and respiration. 1-MCP, an ethylene action inhibitor, maintains quality traits, such as flesh firmness, whereas it reduces volatile production in apple fruit. 1-MCP also affects several storage disorders of apple fruit. 1-MCP treatment inhibits some disorders, such as superficial scald, while 1-MCP increases the incidence of other disorders, such as CO_2 injury and flesh browning. Metabolomics, a comprehensive analysis of metabolites, has recently been applied to the evaluation of various postharvest processes. The effect of 1-MCP on metabolic events depends on several factors; e.g. the cultivar in question, degree of maturity, storage temperature, and atmospheric conditions [1–4]. In this study, we performed NMR-based metabolic profiling of 1-MCP-treated and -untreated Japanese apples. Two cultivars of apple fruit ('Fuji' and 'Orin", the most popular cultivars in Japan) were harvested in Aomori Prefecture, treated with 1-MCP and stored in ambient air at 0°C for up to 8 months. The 1H-NMR spectra of aqueous extracts of lyophilised pulp or juice were collected and subjected to principal component analysis (PCA). Time-dependent changes in metabolite levels were found in both 1-MCP-treated and untreated Fuji and Orin apples. In Fuji, the time-dependent changes in metabolite levels were markedly greater in the 1-MCP-treated apples than in untreated fruit. Sorbitol, malic acid (MA), asparagine (Asn), and aspartic acid (Asp) were relatively abundant in 1-MCP-treated fruit, whereas methanol (MeOH) and ethanol (EtOH) were abundant in the untreated apples. 1-MCP treatment was reported to induce changes in metabolite levels similar to those reported here, which increase the incidence of flesh browning [3], a storage disorder common in Fuji apples. In Orin ap ples, MA, Asn, and Asp were abundant in 1-MCP-treated apples; by contrast, sorbitol, EtOH, and xylose (Xyl) were abundant in the untreated apples. The time-dependent changes in metabolite levels were not ameliorated to any great extent by 1-MCP treatment. In 'Granny Smith' apples, 1-MCP treatment inhibits time-dependent changes in levels of terpenoids, including farnesene-related volatiles [4]. Since 1-MCP treatment inhibits both superficial scald development in 'Granny Smith' [4] and Orin storage, our results suggested that in some cases this disorder is not be associated with changes in aqueous metabolite levels. The effect of 1-MCP on apples may be related to the cultivar in question, maturity at time of 1-MCP treatment, or the storage conditions used. Further studies of the relationship between metabolic events and changes in apple fruit quality are in progress.

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[2] Bekele et al., Physiol. Plant., in press (2015).
[3] Lee et al., Metabolomics, 8 (2015).
[4] Rudell et al., J. Agric. Food. Chem., 57, 8459-8466 (2009)

Keywords: *NMR-based metabolomics, apple, 1-methylcyclopropene*

Acknowledgement: *The project of Ministry of Agriculture, Forestry and Fisheries, Japan, "Development of non-destructive technology evaluating various qualities of agricultural products"*

G8
EFFECT-BASED METABOLOMIC PLASMA PROFILING OF BOVINE RESPONSES TO GROWTH PROMOTING AGENT EXPOSURE

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Despite the EU prohibition of anabolic substances administered to food producing animals, reports suggest that the use of growth promoting agents continues, pursued by improved animal health and subsequent meat quality. Current detection methods have shown erroneous results in detection of the misuse of corticosteroids such as prednisolone and dexamethasone and are unable to discriminate the use of 17β-oestradiol from endogenous levels present in cattle. With advancements in technology the opportunity of discriminating xenobiotic exposure by focusing on the metabolic response of the animal promises longer detection windows with increased sensitivity. In this way we unveil metabolite markers altered in bovine plasma significant to the use of oestradiol, dexamethasone or prednisolone at levels known to encourage increased growth. Twenty-four male beef cattle were randomly assigned to four groups (n=6) for experimental treatment over 40 days; a control group o f non-treated cattle, and three groups administered 17β-estradiol-3-benzoate (0.01 mg/kg per i.m), dexamethasone sodium phosphate (0.7 mg/day per os), prednisolone acetate (15 mg/day per os), respectively. Plasma collected from each animal at day 25, were prepared in triplicate and analysed by ultra-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (UPLC QTof-MS) system where data was collected in continuum mode from positive electrospray ionization after reversed phase separation. Data processing was performed via Progenesis software and based on a plasma fingerprint of 751 ions, multivariate analysis was applied to generate descriptive and predictive models to discriminate treated from untreated cohorts. Results demonstrated separation of control animals from all treatment groups based on 55 ions of interest. Subsequent comparison of the various treatment groups generated robust models for oestradiol, dexamethasone, and prednisolone administrations attributed by 40, 34 and 43 metabolite modifications respectively. Putative identifications were made for the metabolites of interest in order to elucidate the biological mechanisms involved in the endogenous action of growth promoter activity. Further statistical analysis by t-test comparison of treated to control animal cohorts showed 10 metabolite markers significantly altered by all treatment groups, which may find use as effective markers in screening. In a time where the integrity of the meat industry has come under much scrutiny, the applicability of such omics-based screening approaches that can detect drug misuse and ensure safe food consumption is considered highly relevant.

Keywords: *cattle, estradiol-17β, corticosteroid, metabolomics, biomarker*

G9
PROFILING OF METABOLOMIC CHANGES INDUCED BY TESTOSTERONE ESTERS IN PIG PLASMA AND URINE

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The use of hormones as growth promoters for fattening purposes in livestock has been banned in the European Union since 1988 by Council Directive 93/22/EC. However, this banned substances are still reported within the framework of European monitoring residue plans. In order to improve reliability of the detection methods, metabolomics approaches to non-targeted screening for detection of anabolic practices using the naturaly steroid hormones or new synthetic growth promoters have been designed recently [1]. In this study, metabolic fingerprinting to discriminate between pigs treated with 17β-testosteron esters and control animals has been investigated. Twelve 90 day-old pigs were randomly separated into test (8 animals) and control (4 animals) groups. Animals from the test group were treated with i.m. administration of the hormonal preparation (testosterone propionate, testosterone phenylpropionate, testosterone isocapronate, testosterone decanoate; Sustanon 250, N.V. Organon, CZ Reg.56/357/91-C). Urine samples were collected in both groups from 14 to 90 days after treatment. Plasma samples were collected from day 1 to day 90. All pigs were weighed every week within the experiment. Samples were filtered on centrifugal devices to remove proteins and obtained filtrates were mixed with internal standard (testosterone-D3 in methanol, Sigma-Aldrich). Each sample was injected into chromatographic system using an Accera 1200 Series on a Hypersil Gold C18 column for separation. LC-HRMS metabolomic fingerprints were acquired on Q-Exactive mass spectrometer (Thermo Fisher Scientific) in ESI+ mode. Full scan mass spectra were acquired from 80 to 800 *m/z* using a mass resolution of 70.000 FWHM in centroid mode. Raw data were processed by SIEVE and XCMS software. PCA and OPLS–DA were carried out using SIEVE, XCMS and SIMCA-P+ statistic software. The chromatographic analysis of the fingerprints generated 968 ions and 692 ions for plasma and urine, respectively. Statistical evaluation selected only significant peaks (p-value), 374 and 264 peaks were extracted for plasma and urine samples, respectively. Multivariate statistical analysis showed significant metabolic differences between test and control groups on day 28 after aplication of the testosterone hormonal preparation. However, no significant differences between treated and control group were found after day 42. Moreover, the urine samples were examined by conventional target LC–MS/MS. In this case, no testosterone residues were detected after day 14. The study showed also higher growth performance ($p<0.05$) in treated pigs in comparison to control animals, demonstrating anabolic effect of testosterone esters. The work is going on, additional experimental animals will be used to confirm the fingerprinting data to the control of banned testosterone preparation in pigs.

[1] Pinel G. et al. (2010): Targeted and untargeted profiling of biological fluids to screen for anabolic practices in cattle. Trends in Anal.Chem., 29.

Keywords: *metabolomic, anabolic practices, testosterone, pigs, urine*

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G10
METABOLIC PROFILING OF FOOD MATRICES: IDENTIFICATION OF POTENTIAL MARKERS OF MICROBIAL CONTAMINATION

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Bacterial metabolites represent the final downstream products of the genome, thus reflecting the operation of the biological system that is its phenotype. The analysis of metabolic patterns and changes in the metabolism in food along the storage phases has been proven to be an essential tool to the early identification of contaminated products. Current microorganisms' detection methods rely on accurate and sensitive yet time-consuming techniques which provide retrospective values for microbial contamination. The research aims to generate an early warning system able to highlight, in real time, non compliances or contamination of food – meat matrices – within the shelf-life and to provide information which could support companies in accepting or rejecting batches. Analysis are focused on the research of volatile organic compounds (VOCs), generally produced by contaminated food matrices spiked by specific bacteria (Salmonella spp, Campylobacter spp e Stap hylococcus aureus), considering that the spoilage potential of bacteria is mainly related to their proteolytic activity on food.

Keywords: *foodomics, salmonella, campylobacter, VOC, GC-MS*

G11
A MULTI-OMIC APPROACH TO REVEAL THE EFFECT OF LOW-LEVEL GAMMA RADIATION ON RICE SEEDS

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The exposure of plants to ionizing radiation (IR) is known to trigger a wide range of responses between initial absorption of energy and final biological injury. In this study, we performed multi-omic study to investigate the effects of gamma radiation on seeds of rice plants (cv. Koshihikari) grown in radionuclide contaminated soil at Iitate farm located in Iitate village consequent to the nuclear power plant disaster of March 2011, Fukushima prefecture. Seeds from rice plants (cv. Koshihikari) grown in clean soil served as control. The gene expression analysis was conducted using rice 4 × 44 k microarrays and metabolite analysis was done by using LC/MS and GC/MS followed by combined data analysis using GeneSpring software. Gene expression microarray analysis revealed 1891 and 440 genes as gamma ray inducible and repressible genes, respectively (P<0.05). The metabolite analysis using LC/MS and GC/MS revealed 50 differential metabolites (P<0.05). The combined multi-omics analysis revealed modulation of several metabolic and defense pathways related to stress response of plants. Interconnectivity between up-stream and down-stream pathways at gene and metabolite levels were also observed. Our results suggest that the rice plants grown up in radionuclide contaminated soils form seeds with elevated capability to defend well by eliciting appropriate stress responses.

Keywords: *rice, gamma radiation, multiomic analysis, genomics, metabolomics*

GENERAL FOOD ANALYSIS

(H1 – H45)

H1
NUTRITIONAL AND ANTINUTRITIONAL PROFILE OF BORASSUS AETHIOPUM (AFRICAN PALMYRA PALM) SHOOTS

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Borassus aethiopum (African palmyrah palm) shoots was analysed for its nutritional and antinutritional compositions. The proximate composition showed 56.33% *w/w* moisture, 11.2% DW crude fibre, 6.9% DW crude protein and 81% DW available carbohydrate. Mineral content (per 100 g dried sample) indicates the presence Mg (640 mg), Ca (433.3 mg), K (236.7 mg), Mn (12.85 mg), Zn (12.74) and Fe (11.5 mg/100 g DW) as the most abundant. The level of toxic Pb and Cd in the shoot is of great concern considering their health effect. Amino acids analyses showed that the shoot is not a good protein source when compared to WHO/FAO/UNU reference standard for school children. Nevertheless, the shoot contain an appreciable amount of essential amino acids (lysine, threonine, phenylalanine and tyrosine) above the reference standard for adult. The concentrations of hydrocyanic acid, nitrate, oxalate and phytate were lower than the reference toxic standard level. The results indicate that the B. aethiopum shoot is a food stuff with appreciable levels of both macro and micro nutrients as well as safe levels of antinutritional factors.

Keywords: *palmyrah palm, borassus aethiopum, nutrition, antinutritional, wild food*

H2
COMPARATIVE ANALYSIS OF TRADITIONAL DESI GHEE WITH PROCESSED EDIBLE OILS AND SPREADS USING RAMAN SPECTROSCOPY

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Keeping in view the importance of dietary fats in modulating disease risk, a study was planned to compare edible oils, spreads and desi ghee based on fatty acid composition through Raman spectroscopy. The double bonds in unsaturated oils tend to react more with oxygen causing oxidative stress in living cells, therefore the excessive use of processed vegetable oils may pose riskfor human health. In the spectral analysis, Raman peaks at 1063 and 1127 cm^{-1} represent out of phase and in phase aliphatic C–C stretch for saturated fatty acids. The peak at 1302 cm^{-1}, labeled for alkane, decreases with increase in the double bond contents (unsaturation). Further the Raman peak at 1655 cm^{-1} showed a monotonic increase as a function of unsaturation. The double bond contents in the Raman spectra from 1650-1657 cm^{-1}represent unsaturated fatty acids that changes during the synthesis of spreads and banaspati ghee. Desi ghee, extracted from cow and buffalo milk, showed distinc tive Raman peaks at 1650 and 1655 cm^{-1} which originates due to isomers of conjugated linoleic acid (CLA). These Raman shifts differentiated desi ghee from other artificially produced banaspati ghee, spreads and oils. CLA has proved to be anti-carcinogenic, anti-inflammatory and anti-allergic properties, therefore the limited use of desi ghee may reduce the risk of cardiac diseases. Principal component analysis (PCA) has been applied on the Raman spectra that clearly differentiated desi ghee, mono-unsaturated extra virgin olive oil (EVOO) and EVOO spread from other oils, oil mixtures, spreads and ghee.

Keywords: *Raman micro-spectroscopy, principal components analysis (PCA), fatty acids, edible oils, desighee*

H3
APPLICATION OF HIGH PRESSURE LIQUID CHROMATOGRAPHY COUPLED WITH CORONA CAD TO QUANTIFICATION OF NATURAL AND ADDED SUGARS IN VARIOUS FOOD PRODUCTS

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Added sugars are sugars (e.g. sucrose, fructose, glucose) and syrups (e.g. glucose syrup, high-fructose syrup) that are added to foods or beverages during processing or preparation. Such products usually characterize with high caloric and little nutritional value. Consumption of added sugars has risen significantly over the past few decades and has negatively contributed to human health.
The aim of this study was to estimate composition of the chosen food products in view of fructose, glucose, galactose, lactose, maltose and sucrose levels and with an emphasis on added sugars content. There was also verified manufacturer's declaration concerning the type of the used sweetener and its content.
Sugars profiles were estimated in breakfast cereals, jams, yoghurts, instant teas and other sweetened beverages using high pressure liquid chromatography coupled with corona aerosol discharge (CAD). The method was validated for linearity, precision and accuracy. It was characterized by wide concentration range (1–150 μg mL^{-1}), good accuracy (96.1–102%) and precision (1.60–5.03%). The method's linearity (regression coefficient R2> 0.999) and repeatability (RSD <5%) were highly satisfying.
The analyzed products characterized by varied concentrations of individual sugars, especially of fructose. Its highest levels were determined in jams (12–33 g/100 g), while natural yogurts did not contain it at all. Instant teas contained the biggest amounts of sugars (82.95 g/100 g), whereas the lowest ones were estimated in breakfast cereals (4.89 g/100 g). There were also found discrepancies between the declared and determined sugars content with some products containing more and some less sugar than the label stated. Such differences might result in misestimating of fructose and other added sugars consumption in the daily diet, thus, it is important to monitor their levels in foods.

Keywords: *sugars, HPLC, Corona CAD, added sugars*

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H4
COLOUR, ANTIOXIDANT AND BIOCIDE PROPERTIES OF PROPOLIS: STUDY OF CORRELATIONS AND GEOGRAPHIC DISCRIMINATION

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In this work the antioxidant capacity, phenolic composition and colour characteristics of 50 samples of propolis coming from Chile (Bio-Bio region, 13 samples) and Spain (Galicia, 13 samples and Castilla y León, 25 samples) were analized and the correlation among parameters were studied. Total phenolic content of methanolic extracts of propolis was determined according to the Folin–Ciocalteu colorimetric method and total flavonoid content of the extracts were determined using the aluminum chloride colorimetric method. Total Antioxidant Capacity was measured using ABTS free-radical scavenging spectrophotometric method as described by Chen et al (2003) using Trolox® as a reference antioxidant and DPPH scavenging activity as proposed by Blois (1958) and expressed as percentage of DPPH scanvenged. The colour was determined on raw propolis samples using a MiniScan XEPlus and CIELab parameters were calculated for the CIE illuminant D65 and 10º standard obser ver conditions. The parameters calculated were: Lightness (L*), redness (a*) and yellowness (b*). Biocide properties were determined using the five-plate screening test that measures the inhibition zone of different microbial strains. The results show a significant and negative correlation between L* and DPPH antioxidant activity, so the paler the colour the lower the antioxidant capacity. Indeed, yellow colour (b*) and total polyphenol (r=-0.361) and total flavonoid content (r=-0.357) and the antioxidant capacity determined both by DPPH (r=-0.412) and by ABTS (r=-0.414) methods. Indeed b* was negatively correlated with the biocide properties of propolis (r=-0.311). Then the yellower the propolis colour the lower their antioxidant and biocide properties. Other significant correlations were the positive correlations between phenolic composition, especially total phenolic content, and antioxidant capacity determined both by ABTS and DPPH methods. Significant positive correlations were also observed between phenolic composition, mainly total flavonoid content, and biocide properties. Regarding geographical discrimination a significant correlation between geographical origin and L*, total polyphenols, total flavonoids, antioxidant capacity determined both by ABTS and DPPH methods and biocide properties were observed, due to the higher L* values and lower phenol contents, antioxidant and biocide properties of Chilean propolis.

Keywords: *colour, antioxidant, biocide, Spain, Chile*

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H5
DIRECT CARBOHYDRATE ANALYSIS IN BEVERAGES AND FOOD USING PULSED AMPEROMETRIC AND CHARGED AEROSOL DETECTION

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Carbohydrates are important food components affecting taste and nutrition. The determination of the types and levels of carbohydrates in foods is important for energy evaluation, nutritional labeling, quality control and for identifying any possibl product adulteration.
Carbohydrate analysis can be problematic since they are very polar compounds, exhibit similar structural characteristics and do not have a suitable chromophore. Their analysis can be performed after suitable derivatization using techniques such as gas chromatography, capillary electrophoresis, HPLC with ultraviolet (UV) or Fluorescence (FL) detection or HPLC with mass spectrometry (MS). These methods utilize derivatization to improve the chromatographic resolution and detector sensitivity; however they can lead to increased assay variability. Direct methods that do not require derivatization include HPLC with refractive index (RI), High-Performance Anion-Exchange Chromatography with Pulsed Amperometric detection (HPAE –PAD) using a high pH mobile phase or HILIC mode chromatography with charged aerosol detection. This poster presents approaches for carbohydrate analysis that solve the challenges for separation and detection of carbohydrate analysis without requirement for laborious derivatization: HPLC with pulsed amperometric detection (HPLC–PAD), HPLC with charged aerosol detection (HPLC–CAD).
When the PAD option on the electrochemical detector is coupled with a gold working electrode, it provides high sensitivity and selectivity for the measurement of carbohydrates in complex food sample matrices. The charged aerosol detector is a mass-sensitive detector that can measure all non-volatile, and many semi-volatile compounds in a sample. The method has a limit of detection of <10ng, on column and a wide dynamic range that covers nanogram to microgram levels with high reproducibility. Application examples for various juice samples, milk and other dairy products, syrup and honey will be discussed.

Keywords: *carbohydrates, foods, beverages, HPLC, pulsed amperometric and charged aerosol detection*

H6
MECC ANALYSIS OF AMINO ACIDS IN RAPESEED PROTEIN FRACTIONS FROM GENTLE BIOPROCESSING IN PILOT PLANT SCALE

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A growing demand for high quality plant protein products for food ingredients has increased the interest in gentle bioprocessing methods preserving the native protein structures and properties suitable for tailoring high quality food. Quantitative and qualitative process control parameters are key to develop robust bioprocessing methods for industrial scale. Important product characteristics of the protein fractions include amino acid profiles to allow formulation of nutritionally optimal food. In addition the presence and composition of free amino acids and small peptides are important parameters, as they often act as bioactives and contribute to sensory characteristics of the protein preparations. Amino acid analyses using high performance capillary electrophoresis (HPCE) allows for rapid and simple analyses with low running costs due to cheap capillaries and minimal buffer use. In this work we have developed an MECC method (micellar electrokinetic capillary chromatography) for separation and quantitation of amino acids, using tetradecyltrimethylammonium bromide (TTAB) and UV detection after derivatisation of amino acids with dinitrofluorobenzene (DNFB). This method is also highly efficient for analyses of protein hydrolysed using reflux in 6N HCl overnight. The MECC method has been optimized with respect to concentrations of TTAB, sodium tetraborate, sodium phosphate and 1-propanol in the running buffer as well as running conditions. Mixtures of standard amino acids were used for optimization resulting in high repeatability and a limit of quantification of 5 µM. The method was employed for analyses of rapeseed protein fractions prepared in pilot plant scale. The rapeseed protein fractions showed differences in protein composition and functional properties, and the fractions were characterised with respect to the presence of free amino acids as well as total amino acid composition using the MECC method developed. Application of MECC for chiral separation of amino acids was employed as an additional quality parameter for evaluation of possible bioprocessing effects on amino acid modification. The profiles of the free amino acids in the various fractions were evaluated as potential markers for protein composition in the fractions to exploit the possibilities for development of an industrially applicable and fast process control method.

Keywords: *MECC, amino acid analysis, bioprocessing, rapeseed protein, DNFB*

Acknowledgement: *PROTEIN2FOOD (H2020-EU.3.2)*

H7
COMBINED CHROMATOGRAPHY AND MASS SPECTROMETRY FOR THE MOLECULAR CHARACTERIZATION OF FOOD EMULSIFIERS

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Food emulsifiers are widely used to stabilise water-fat emulsions such as mayonnaise and dressings. They are prepared by oligomerisation of a poly-alcohol (as e.g. glycerol or citric acid) followed by a reaction with fatty acids or partial acylglycerides. In order to gain insight in the chemical composition of different emulsifiers, a range of chromatographic methods including gas chromatography, size exclusion chromatography, normal phase- and reversed phase liquid chromatography either or not in combination with mass spectrometry was deployed. The different methods turned out to be highly complementary. By applying them in the right order the polar head group and the fatty acid part of the emulsifier can be characterised in detail. Mass spectrometry is indispensable for establishing the number of polar molecules in the head group as well as for establishing the correct combinations of fatty acids in one molecule. Ten commercial emulsifiers were described a t the level of number and type of polar groups and fatty acids present.

Keywords: *fat containing foods, emulsifiers, chromatography, mass spectrometry*

H8
THE INFLUENCE OF ROOTSTOCK, MATURITY AND EXTRACTOR SETTING ON THE CONTENT OF LIMONIN OF PÊRA-RIO ORANGE JUICE

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Brazil is the largest world producer and exporter of orange juice, the most consumed fruit juice in the world. The quality of orange juice is influenced by many factors that affect its sensory and nutritional characteristics. Bitterness has a negative impact on orange juice. Limonin, the main responsible for bitterness in orange juice, is not present in the in natura fruit, but the tasteless precursor limonoate A-ring lactone, which is converted into limonin when fruit tissues are disrupted during extraction. The aim of this work was determine limonin in orange juice of Pêra Rio variety in order to evaluate the influence of rootstock, maturity and extractor setting on the limonin content of the juice. For extraction of limonin, orange juice was heated, centrifuged and passed through a SPE C18 cartridge. Then it was washed with water, eluted with acetonitrile and filtered. The extraction was performed in triplicated. Chromatographic analyses were performed i n a Shimadzu LC-20A using a C18 column. The mobile phase consisted of a linear gradient program beginning at 30% acetonitrile and 70% water and finishing at 45% acetonitrile and 55% water in 30 min, at 210 nm. For quantification external standard method was used. Each extract was analyzed in duplicate. Standard of limonin was used to compare the corresponding retention time, mass spectrum and extracted ion chromatogram from orange juice extract. Limonin was identified in the orange juice extracts as the peak at 26.8 min, which showed the same retention time of the limonin standard solution. The identity of limonin in the orange juice extract was confirmed by mass spectrum of the peak at 26.8 min, which matched that mass spectrum of the peak of the limonin standard. Also the extracted ion chromatogram for m/z 471.2 (positive ion mode) of the orange juice extracts confirmed the peak at 26.8 min as limonin. Limonin was determined in Pêra Rio orange juice from 'Cleopatra' m andarin and 'Rangpur' lime rootstocks extracted at the FCOJ and NFC settings during the 2013 harvest (four collections periods, July to October). The level of limonin ranged from 0.86 to 3.94 μg.mL-1 orange juice. It was observed a reduction in the limonin level of Pêra Rio orange juice during maturation. The results indicated that there was a greater reduction in limonin of orange juice from the NFC setting than the FCOJ one. Orange juice from 'Rangpur' lime rootstock extracted with the usual setting for FCOJ and NFC showed a more pronounced fluctuation of limonin level in the beginning of harvest. Also, it was observed that bitterness remained more in the juice from 'Rangpur' lime than that from 'Cleopatra' mandarin rootstock. PCA showed that limonin differentiates juices from different rootstocks in the beginning of harvest, while at the end of harvest with ripe orange, juices were discriminated according to the extraction setting based on limonin, solub le solids and total sugars.

Keywords: *limonin, orange juice, HPLC, bitterness, PCA*

Acknowledgement: *This work was supported by PADC/FCF/UNESP and the São Paulo Research Foundation (Fapesp), 2013/10.138-0 and 2014/23.303-1.*

H9
NUTRACEUTICALS IN BLOOD ORANGE JUICE AND BY-PRODUCTS

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The global nutraceutical market is expected to reach $ 241.1 billion by 2019, for this reason the characterization of biomolecules in food and its industrial waste is actually a topic of great interest. As previously reported Citrus fruits and the resulting industrial products and by-products are rich in bioactive compounds [1,2]. These can be recovered and re-esed in different fields, such as consmetical, food and feed industries. Within our research activities we recently focused our interest on the characterization of biomolecules in citrus derivatives. The present study reports on the characterization of flavonoids, polymethoxilated flavones, and anthocyanins, and for the first time limonoids, determined in the individual parts of blood oranges and in all the resulting industrial by-products. Most blood oranges come from Mediterranean countries (Sicily in particular) and are particularly appreciated for the high amounts of anthocyanins and the characteristic flavor of the juice. The samples analyzed were blood orange juice and the by-products resulting from the entire industrial process, namely waste water, peels, seeds, pulps (obtained by centrifugation and by decantation), and on untreated dried solid residue and the calcium carbonate treated dried solid residue. Depending on their chemical classes, biomolecules were extracted by different procedures optimized to achieve excellent recovery values for all the analytes (85–92%). Analysis of polymethoxyflavones and anthocyanines were performed by HPLC–PDA. Limonoids and polyphenols were analyzed by HPLC–PDA-MS using both APCI and ESI interfaces. Decanted pulps resulted to be the by-product richest in bioactive molecules (136.4 mg/g), with the greatest concentration of flavonoids (130.6 mg/g). The highest content of polymethoxyflavones and limonoids was found in the calcium carbonate treated dried solid residue (0.4 mg/g) and in seeds (10.0 mg/g) respectively. The results obtained suggest that blood oranges contain high amount of several nutraceutical compounds, therefore the industrial by-products could be used for their recovery.

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Keywords: *limonoids, blood orange, by-products, HPLC, nutraceuticals*

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H10
PULSED AMPEROMETRIC DETECTION AT GLASSY CARBON ELECTRODES: A NEW DETECTION SCHEME FOR SENSITIVE AND REPRODUCIBLE DETERMINATION OF POLYPHENOLS AND BETA-AGONISTS IN LIQUID CHROMATOGRAPHY

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Since the first papers by Johnson and La Course [1] in the 80's, pulsed amperometric detection (PAD) at metal electrodes has been exploited for the sensitive and reproducible determination of several compounds. On the contrary, carbon-based electrodes are used at constant potential (DC), sometimes modified in order to improve the response [2.3]. Under DC detection GCEs show fouling problems due to the strong adsorption onto the surface of sample matrix components and/or analyte oxidation products. This process leads to a decrease of the sensitivity and a time-dependent deterioration of the response, therefore an electrode regeneration is required at the beginning of each working session. Indeed, starting from the 1980s these problems have been already evidenced, and surface off-line preactivation [4,5] or in-situ laser irradiation [6] procedures were found necessary to obtain reproducible and well-defined electrochemical behaviors. Recently, we have demonstrated [7] the proof of concept of the PAD at GCE operating in typical mobile phases containing organic solvents, thus overcoming problems of the activation procedures. In this context, when the electrochemical detection is coupled with liquid chromatography, the proper choice of the mobile phase is a fundamental aspect, since the detection conditions have to be tuned with those of chromatographic separation. In the present work the new detection scheme based on PAD at GCEs and operating in typical chromatographic mobile phases for the sensitive and reproducible detection of polyphenols and beta-agonists is described. Preliminary experiments by cyclic voltammetry were carried out to investigate the electrochemical behavior and to select the detection and cleaning electrode potentials. The proposed potential-time parameters, including detection and delay times, were optimized in terms of sensitivity, limit of detection and response stability. The optimized waveform allowed the sensitive and stable detection of the analytes at ppb levels, with precision values under repeatability conditions ranging from 3.0 to 5.9%. The potential of the new detection method has been also tested in liquid chromatography by the determination of polyphenols and beta-agonists in complex real samples.

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Keywords: *pulsed amperometric detection, glassy carbon electrode, Beta-agonists, polyphenols, high performance liquid chromatography*

H11
CURRENT ADVANCES IN INSTRUMENTATION OF DUMAS TYPE PROTEIN ANALYZERS

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N-protein analysis based on the Dumas principle relies on quantitative conversion of the sample of interest into well-defined gaseous species at elevated temperatures in the presence suitable reagents and elemental oxygen. Excess oxygen is commonly bound on metals such as copper or tungsten, so-called reducing metals, prior to chromatographic separation of the combustion gases followed by detection of N_2 correlating to protein content. Copper or tungsten react to the respective metal oxide throughout the course of several hundred analyses causing deficiency in oxygen binding as well as insufficient conversion of NO_x to N_2. Thus, erroneous nitrogen / protein values result. In order to circumvent such effects most manufactures of Dumas-type analyzers propose frequent exchange of reducing metals. Commonly such metals are a key factor in terms of price per analysis. A unique method to recover the metal oxide to the active metal species increasing life time and d ecreasing cost per sample is presented. Experimental results depict a decrease in price per analysis of up to 50% depending on the manufacturer and an approx. fivefold increase in life time of the reducing metal. Moreover, experimental results on utilizing argon instead of helium as carrier gas are presented. Argon proved to be suitable for the majority of applications in full accordance with industry relevant standards.

Keywords: *Dumas, protein determination, elemental analysis, N protein analysis*

H12
SHELF-LIFE EVALUATION OF READY-TO-EAT DESSERTS

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Currently, consumers are increasingly becoming more demanding regarding the quality of food, holding high expectations from the time of purchase to the consumption of foodstuffs. Thus, food should be safe and sensory alterations should be minimized. The shelf-life of most perishable foodstuffs is based on the study of the survival and growth of microorganisms. However, this study is insufficient to assure the quality and safety of food, making it necessary to carry out physiochemical and sensory analysis to ensure the final quality of products. The objective of this study was to characterize the physiochemical, microbiological and sensory properties of four ready-to-eat (RTE) Portuguese desserts: “Brigadeiro”, “Brigadeiro Branco”, “Beijinho” and “Torta de Limão”, during storage time. The desserts were stored for 30 days at a refrigeration temperature of 4±2°C The aim of the work was to establish the shelf-life and produce a label with the nutritional composition of each RTE product. Throughout the study, the following physiochemical parameters were determined: pH, aw, moisture, minerals, protein, carbohydrates, fat, fiber, chlorides, total sugars and the instrumental texture (hardness and adhesiveness). Results showed a slight increase of pH, moisture, hardness and adhesiveness during storage time. The microbiological evaluation included the determination of: *Salmonella spp*., enumerations of *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive *Staphylococci, Listeria monocytogenes* and total viable counts (TVC) (microorganisms at 30°C). It was concluded that the different RTE desserts comply with the parameters of quality and food safety required by Regulation (CE) Nr. 1441/2007, according to the guidelines set by the Health Protection Agency (HPA) and the Institute of Food Science and Technology (IFST). Sensory analysis was performed according to a semi-trained panel that evaluated the following attr ibutes: appearance (color, brightness, crystal surface, water surface and the presence of mold), odor (characteristic (sweet), acid, rancid and strange), texture (firmness, adhesiveness, dissolution, presence of sugar crystals, hardness, watery) and flavor (characteristic (sweet), acid, fermented). The panel did not detect differences up to 8 days of storage time.

Keywords: *shelf-life, ready-to-eat, desserts*

H13
REQUERIMENTS OF TOOLS NECESSARY FOR QUALITY AND SECURITY CONTROL OF DAIRY COOPERATIVES

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Dairy industry carries out quality controls for guarantee its products security. These types of controls are based on the current legislation where the sampling and test allowed are defined to be done before milk entry or liberation. The testing analytes method on milk is chosen depending of many factors. New tools are being researched as alternative or improve version of current but it development involves different stages.

BIOFOS project is focused on approach an innovative methodology supplying with the development of a sensor. In order to claim the success of it a strengthen data exchange between developers and potential end-users is required.

Manufacturers are interested about new, easy-use and cheap tools, offering quick and reliable results. In order to obtain all the end-users requeriments and the potential integration scene, two ways of collecting information have been used:

1. Surveys: their structure was divided in four parts:
- Introduction about the cause of the survey
- Collecting contributor information
- Collecting information about tests and controls routines
- Brief explanation of BIOFOS sensor and collecting impression and suggestions

Surveys were distributed to vet services, research centers, labs, manufacturers and farmers. A total of 35 surveys were filled. They have allowed the main specifications and specific requeriments identification:

Costs: <0.5 € per analysis
Time required to results: < 5´
Reusability: 30 times
Analytes: AFM1, penicillin, tetracycline
Utility of the sensor: 5x10x10 cm sensor size, 12 hours of autonomy.
Wireless results transfer option available.
Detection limits AFM1 <25 ppb, Penicillin-4 ppb, Lactose <0. 01%

Dairy chain review: it has allowed the knowledge of quality department planning focused on the different control points:
- Cows and tank sampling on farms: AFM1/antibiotics. AFM1 is the metabolized form of AFB1 from grains, farmers could be more interested of feed supplier control. In parallel, if illness occurs on farms, farmers provide antibiotics to cows. They could detect with a quick sensor if the suppression period has been completed with certainly or when any doubt about persistence of residues after treatments occurs.
- Trucks reception and control of process on industry: AFM1/lactose.

AFM1 is controlled during tank reception from farms. When new farms or external milk suppliers have to be joined, more accurate controls should be done.

Some dairy industries produce lactose-free products by lactase enzyme. In order to guarantee the lactose-free label specification is achieved, final products are tested before their liberation.

The main interests of potential end-users have been:
- Price
- Rapidity
- Analytes
- Ease
- Reusability

In conclusion, BIOFOS sensor combines with quality controls of the dairy industry. The activities achieved have shown that antibiotics-treated cows´s milk is a strategic detection for farmers. AFM1 + antibiotics detected in the same test for quick screenings could serve for simplifying sampling. Lactose detection with an on-line application would contribute on facilities would be required to its usefulness and success increase.

Keywords: *dairy, quality control, sensor, food security, industry*

Acknowledgement: *EUROPEAN COMMISSION*

H14
NUTRITIONAL COMPOSITION OF WATER LILY (NYMPHAEA LOTUS) RHIZOME FROM RIVER RIMA, SOKOTO STATE, NIGERIA

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The proximate, minerals, amino acids and anti- nutrients compositions of Water lily (*Nymphaea lotus*) rhizome were evaluated. The proximate analysis revealed the following: moisture 48.83%, ash 4.67%, crude lipid 1.83%, crude protein 4.23%, carbohydrates 35.44%, crude fiber 5.00 and energy value of 742.10 kcal/100g on dry weight basis. Magnesium (840.00 mg/100g), calcium (666.67 mg/100g), potassium (416.67 mg/100g), and phosphorus (272.88mg/100g) were the predominant macro elements present in the rhizome. Iron (31.58 mg/100g), manganese (3.45mg/100g) and zinc (3.27 mg/100g) were the micro elements detected in appreciable amounts. Essential amino acids were above the recommended level by Food and Agricultural Organization/World Health Organization (FAO/WHO) for adults. The results of anti-nutrients to nutrients molar ratios are below the critical levels known to inhibit the availability of some minerals element. The present investigation showed that water lily (*Nym phaea lotus*) rhizomes are rich source of many important nutrients that appear to have a very positive effect on human health.

Keywords: *water lily (Nymphaea lotus) rhizome, minerals, amino acids, anti-nutrient agents*

H15
OCCURRENCE OF HISTAMINE IN FRESH FISH COMMERCIALLY AVAILABLE IN POLAND

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Biogenic amines, including histamine, belong to compounds that can affect human health. Histamine naturally occurs in many species of fish with dark meat, especially of the families: Scombridae, Clupeidae, Engraulidae, Coryfenidae, and Pomatomidae. High concentration histamine in fish and fish products may cause food poisoning in humans. Scombrotoxic fish poisoning (SFP) is associated with the consumption of contaminated fish of the Scombridae family, for example: tuna, mackerel and herring. This intoxication causes cardiovascular, gastrointestinal, and neurological symptoms, such as skin rashes, urticaria, oedema, local inflammation, nausea, vomiting, diarrhoea, cramping, hypotension, headache, palpitation, and oral burning. The symptoms usually resolve within 24 h. During the food intake process in the human gut, low amounts of histamine are metabolised to a physiologically less active degradation products. However, upon intake of much histamine with foods, the detoxification system is unable to eliminate histamine sufficiently. The high performance liquid chromatography with diode array detection (HPLC–DAD) is the reference method in accordance with Commission Regulation No. 2073/2005. In the present study, a total of 132 samples of fresh fish were collected from various local shops in Pulawy region (south-east of Poland). The specimens included fish such as mackerel, herring, cod, salmon, brown, trout, pollock, flounder and sprat. After purchasing, the samples were immediately delivered to the laboratory at refrigerated temperature. After processing of examined fish samples, histamine was detected with HPLC–DAD analysis with the 1.3 mg/kg. Histamine was detected in 18 out of 132 samples (13.6%): sprat (7 samples), cod (3 samples), herring (3 samples), salmon (3 samples), flounder (1 sample), and roach (one sample). The concentration of histamine ranged between 1.4 and 86.7 mg/kg. The maximum amount of histamine (86.7 mg/kg) was found in the salmon. In 86.4% of the examined samples no histamine was found and in 13.6% fish tested the concentration of histamine was below the allowable limit (100 mg/kg). The study showed that fresh fish meet the food safety criteria for histamine listed in the Commission Regulation (EC) No. 2073/2005. The low content of histamine in fish and fish products is an indicator of freshness and shelf life, which shoves that fish were fresh, as raw materials, and manufacturing process was carried out correctly. The results obtained demonstrated that fresh fish available on Polish markets are safe for the consumers.

Keywords: *fresh fish, histamine, HPLC-DAD*

H16
OFFICE CHROMATOGRAPHY: PRECISE SAMPLE APPLICATION ON MINIATURIZED PHASES, IMAGE EVALUATION AND DART-MS SCANNING

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Office chromatography combines new achievements in office technologies with future trends in planar chromatography to an userfriedly all-in-one system. The precise application of samples on sensitive miniaturized or nanostructured layers is a new challenge. These ultrathin phases tolerate only a minimum of solvent and therefore a thermal ejecting Bubble Jet printer is suitable for exact sample application after technical modifications [1]. These modifications include the removal of unnecessary parts of the printing unit and a plate guide system for save and precise handling of the sensitive layers. The original cartridge system was replaced by filter vials for small sample volumes and for handling solutions with different viscosities. The purge unit was improved to avoid sample diversion. The application range of the print head was determined for different application modes. A rational application range of the print head was determined to be 5–50 nL per printjob depending on application area and resolution. Such low nanoliter applications were used to deposite different samples on miniaturized thin-layer substrates. The reproducibility (%RSD) for printing was proven to be 2.9–7.2% and the trueness was determined by mean recovery rates of 101–105%. Quantitative determinations were performed by a high-resolution scanner and picture evaluation software. Additionally, desorption-based mass spectrometry was studied on the ultrathin layers [2]. Finely graduated scales of three preservatives were printed to determine the spatial resolution of scanning Direct Analysis in Real Time mass spectrometry. A full width at half maximum (FWHM) of 0.8 mm was determined for the spatial resolution of an optimized scanning DART–MS interface [3]. Office chromatography provides several advantages such as very low reagent consumption by a clean, versatile and cheap working station including all automated chromatographic and detection steps. It is a modern concept for online planar chromatography using miniaturized layers as a core for an end-user friendly system

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Keywords: *miniaturization, high-performance thin-layer chromatography, ultrathin-layer chromatography, bubble jet application, DART-MS*

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H17
ANALYSIS MONO AND DISACCHARIDES IN BEVERAGES USING LC WITH MASS DETECTION

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Sugars and sugar alcohols are classes of carbohydrates that are important in human nutrition and natural constituents of foods. With the increasing incidence of obesity and diabetes across the developed world, interest in monitoring sugar intake has vastly increased in recent years. Consequently there are now requirements to provide accurate information on product labelling in order to comply with increasingly stringent regulatory demands. Profiling the sugar content of products is also a useful tool in assessing authenticity of products and to detect adulteration. The analysis of sugars and sugar alcohols remains a challenging application, owing to the lack of chromophores and the similarity between these molecules, many of which are isomers of one another. Due to its separation power, accuracy and speed of analysis, HPLC has become the method of choice for the analysis of sugars. An alternative to RI and ELS detection is the use of mass detection with electrospray ionization (ESI). Mass detection is complementary to traditional detectors used for LC. It offers the opportunity to improve detection limits and also to obtain mass spectral information on the components in the sample. The combination of both chromatographic retention time and mass information results in increased selectivity for the analysis of sugars and sugar alcohols. Here we show the application of the ACQUITY QDa Detector coupled to the ACQUITY Arc system for the profiling and quantification of sugars in juice, wine, beer, cider and whisky.

Keywords: *sugars, UPLC, QDa*

H18
ANALYSIS OF MONOSODIUM GLUTAMATE IN FOOD FLAVORING POWDERS USING PRE COLUMN DERIVATISATION AND MASS DETECTION

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Monosodium glutamate is the sodium salt of the non essential amino acid glutamic acid and is added to various food products as a flavor enhancer. In this poster a method for indicating and quantifying the presence of monosodium glutamate (MSG) in sachets of food flavoring powder is summarized. Samples of food flavoring powder as found in instant noodle based products are extracted with water and derivatised with AccQ-Fluor reagent followed by analysis on liquid chromatography coupled with a compact mass detector. A selection of flavouring powder samples were analysed, including products with declared MSG content and products which did not indicate MSG was added. Excellent sensitivity, selectivity and repeatability will be presented with linear calibration over a suitable concentration range.

Keywords: *MSG, QDa, UPLC, AccQFluor*

H19
MONITORING OF MICROBIAL CONTAMINATION IN READY-TO-EAT PRODUCTS IN SOUTH KOREA

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The purpose of this study was to investigate the microbiological contamination such as total aerobic bacteria, coliforms, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens and Vibrio parahaemoliticus in ready-to-eat products. Ninety-five samples of ready-to-eat food, 40 samples of fresh-cut produce (vegetables and fruits), and 80 samples of ready-to-eat seafood (fresh and frozen) were collected according to the sampling plans recommended by The International Commission on Microbiological Specifications for Foods (ICMSF) from randomly selected grocery stores and markets in South Korea. Aerobic bacteria were found at the levels of Not Detected (ND)~4.4 log cfu/g, ND~6.3 log cfu/g and ND~5.8 log cfu/g in ready-to-eat food, fresh-cut produce and ready-to-eat seafood, respectively. Coliforms were found at the levels of ND~3.3 log cfu/g, ND~4.9 log cfu/g and ND~3.6 log cfu/g in ready-to-eat food, fresh-cut produce and ready-to-eat seafood, respectively. E. coli, S. aureus, C. perfringens, B. cereus and V. parahaemoliticus were not found in any samples. The monitoring results could be used to improve the standards and specification in Korea Food Code and to harmonize with the international standards.

Keywords: *microbiological contamination, ready-to-eat food, fresh-cut produce, ready-to-eat seafood, ICMSF*

Acknowledgement: *This research was supported by a grant, 13161MFDS004, 2015 from the Ministry of Food and Drug Safety, South Korea*

H20
DEVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF HISTAMINOL, HYDROXYTYROSOL, TYROSOL, AND TRYPTOPHOL IN WINE

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The conversion of amino acids is an important topic in the field of fermented foods, like wine. Amino acids together with proteins and peptides play an important role as nitrogen sources for yeast and lactic acid bacteria, respectively, during alcoholic and malolactic fermentations. Biogenic amines and alcohols represent the two main molecular groups through which the starting amino acids are converted. Biogenic amines are produced by microbial decarboxylation of the corresponding amino acid precursors. These bioactive compounds in wine play an important role, because not only the high levels of biogenic amines intake can lead to a variety of human symptoms, but also these molecules occurred in wine may cause spoilage. The other catabolic way adopted by microorganisms produces alcohols (fusel alcohols). These compounds derive from amino acids catabolism through the well-known Ehrlich pathway. Yeasts convert amino acids through three enzymatic steps: transami nation to form α-keto acid, decarboxylation to an aldehyde, and reduction to the fusel alcohol. It is known that Saccharomyces cerevisiae can use tyrosine and tryptophan as the only source of cellular nitrogen, the main products of their catabolism being respectively tyrosol, hydroxytyrosol, or tryptophol. These represent compounds of pharmaceutical interest showing several health-enhancing activities, deriving from their free radical scavenging, anticarcinogenic, cardiopreventive and antimicrobial properties. However, concerning a well-known fermented product such as wine, the analysis of the corresponding alcohol derived from catabolism of L-histidine (histaminol) has not been extensively studied even though its presence in wine has been detected by our previous study conducted on a set of several commercial wines. According to the Ehrlich pathway, histidine should be transformed by yeast into a higher alcohol, in this case histaminol. The present method development started by the synthesis of histaminol and hydroxytyrosol (molecules not readily available as a standard), followed by the complete characterization of their chemical structure by ESI–MS and NMR measurements. Then, based on the assumptions described previously, the validation of a LC–MS chromatographic method for the quali/quantitative characterization of histaminol, hydroxytyrosol, tyrosol, and tryptophol in wine has been described and discussed. The four standards showed a good linearity with high correlation coefficient values (over 0.9989) and LOD and LOQ were 0.001–0.015 mg/L and 0.004–0.045 mg/L, respectively. The method was finally applied to detect the target molecules in real samples. In the wines analyzed, the average concentrations of histaminol, hydroxytyrosol, tyrosol, and tryptophol were 0.16, 1.51, 94.01 and 3.89 mg/L, respectively. In this context, a validated LC–MS method may prove to be a valuable analytical support to assess changes of these molecules during the fermentation process.

Keywords: *histaminol, hydroxytyrosol, tyrosol, tryptophol, wine*

Acknowledgement: *This research has been funded by the Università degli Studi del Piemonte Orientale "A. Avogadro" (FONDI DI ATENEO PER LA RICERCA 2014)*

H21
A METHOD TO ASSESS FALSE POSITIVE AND FALSE NEGATIVE RATES WITH REGARD TO RESOLVING POWER, PRECURSOR ISOLATION AND COMPLEXITY OF THE MATRIX FOR HIGH RESOLUTION ACCURATE MASS SPECTROMETRY

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Currently there is a multitude of HRAM methods available, ranging from single stage HRAM-MS to data independent data acquisition (w and w/o precursor selection) to triple quadrupole equivalent methods. All these methods claim improved selectivity, reduced false positives (FP's) and false negatives (FN's) in comparison to the gold standard. But it seems there are uncertainties to what extend the influencing factors change FPR's and FNR's, and consequently, which method or instrument setting is appropriate for the particular experiment. In most cases interferences are the reason for either false positive or false negative results. A mass spectrometric interference could cause both FN's and FP's. A FN is observed, if the true mass centroid is shifted outside the extraction window by a non-resolved interference. In contrast, FP's occur, when a signal originating from a different compound is measured within the mass extraction window. Therefore these cases have to be evaluated separately from each other. However, for both resolving power and amount of potential interference (controled by precursor isolation) are determining factors. In this study we examine interferences with computational methods. Effects of mass spectrometric resolving power and precursor isolation window are shown.

Keywords: *high resolution accurate mass, selectivity, pesticides, veterinary drugs, residues*

H22
FACTORS AFFECTING OVERFOAMING OF BEER (GUSHING)

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Gushing, a phenomenon observed in many saturated beverages such as beer, is characterized by a massive overfoaming of a drink upon a container opening. The principle of gushing is a sudden conversion of carbon dioxide dissolved in the drink to a gas. Gushing occurs only when a sufficient degree of supersaturation of the solution with carbon dioxide is reached. Fungal infection of barley and production of simple protein factors, so-called hydrophobins, belong to the most common reasons of gushing. Calcium oxalate from barley is another important initiator of this phenomenon. Gushing is split into two groups: primary and secondary. Primary gushing is caused by fungal infestation of barley and malt and metabolites produced by these them. Gushing of beer thus warns a consumer about the presence of some mycotoxins. Secondary gushing is not directly related to malt quality but it is caused by faults during the brewing process, incorrect handling with beer and substances contained in it (oxalates, high concentration of heavy metals or isomerized hop extracts. Numerous factors responsible for tendency of beer to overfoaming cannot be simply reduced to causes and consequences. The investigation of this complex phenomenon must include microbiological, chemical, and technological aspects, with a special focus on malt and beer production processes under real conditions of malt houses and breweries. In 2014, sampling of intermediates of malt production started in a traditional floor malt house in the Czech Republic. In the collected samples, selected factors that can affect overfoaming of beer (mycoflora, mycotoxins, oxalates, PDMS, methionine, Kolbach index and selected phenolic acids) were studied. The aim of our work is - based on a long-term monitoring of the malting process under real conditions of the malting plant – to find relationships between different causes responsible for overfoaming of beer.

Keywords: *gushing, malt production, mycoflora, mycotoxins*

Acknowledgement: *This study was supported by the project TE02000177 – Center for the Innovative Use and Strengthening of Competitiveness of Czech Brewery Raw Materials and Products.*

H23
PRESENCE OF SULFUR DIOXIDE IN DRIED FRUITS

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Sulfur dioxide is a natural antioxidant agent, and preservative. As such it is also used for the preservation of dried fruit, while it preserves colour and appearance of the fruit and prevents decay.
Sulfur dioxide is on the list of allergens which, according to the directive of the European Regulation, must be clearly and visibly declared to customers to warn of food ingredients that can cause allergies and/or food intolerance, even in very small quantities.
However, food manufacturers are required to declare this additive as allergen only if more than 10 mg/kg or 10 mg/l was added in food, although significantly smaller amounts of sulfur dioxide in individual consumer can cause difficulties
The method used for this determination is ISO 5522. On this occasion, variation in sulfur dioxide content was analyzed during a four year period in samples of dried grapes, dried apricots and dried figs. These are examples where maximum permissible quantity of sulfur dioxide according to the European Regulation is 2000 mg/kg, and as such are a potential risk to consumers.
The test samples were observed in four categories: samples with sulfur dioxide <10 mg/kg, samples having 10–500 mg/kg, 500–2000 mg/kg, and the samples with sulfur dioxide content of >2000 mg/kg.
The number of samples of dried grapes in which the sulfur dioxide <10 mg/kg did not significantly changed over four years while the number of samples with content of 10–500 mg/kg increased from 9.5% to 54.4%, and the percentage of samples with sulfur dioxide content of 500–2000 mg/kg decreased from 38.1% to 11.2%.
In the case of dried apricots the number of samples with the content of <10 mg/kg have decreased over the four years from 23.8% to 0% but the number of samples with sulfur dioxide content of 500–2000 mg/kg increased over the years from 71.4% to 100%.
In samples of dried figs, no significant changes happened in the content of sulfur dioxide over four years.
In conclusion, the fig samples are samples which do not usually contain sulfur dioxide, while the samples of dried apricots are samples who always have contain sulfur dioxide and the highest percentage of these products have a content of sulfur dioxide 500–2000 mg/kg. Such product, if we take into account the daily dose of 0.7 mg/kg of body weight (for the content of 2000 mg/kg) and based on the average body weight of 70 kg, can cause problems to certain consumers, even at the amounts of 25 g.
For samples of dried grapes, the only significant change is the reduction of the samples with the content of sulfur dioxide from 500–2000 mg/kg, but also an increase in sample values with sulfur dioxide between 10–500 mg/kg. This product, if one takes into account the daily dose of 0.7 mg/kg of body weight, (for the content of 500 mg/kg) and based on the average body weight of 70 kg, can cause problems to individual consumers, even at quantities of 100 g.

Keywords: *sulfur dioxide, allergen, dried fruit*

H24
MEASUREMENT OF HIGH DENSITY SAMPLES USING LO-RAY-LIGH® DIFFRACTION GRATINGS IN UV-VIS SPECTROSCOPY

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It has been more than half a century since the release of the first Shimadzu UV-VIS spectro-photometer QB-50 in 1952 and during this time more than 160.000 UV-VIS spectrometers have been produced and installed in a wide variety of different applications. A lot of technical innovations have been implemented to improve the performance and significantly reduce the stray light levels. The latest innovation during development of sophisticated spectrophotometers is based on a new holographic exposure method and optimized etching process which has made it possible to produce both high-efficient and exceptionally low stray light gratings. These LO-RAY-LIGH® gratings have guaranteed values of stray light at the intermediate position between zero- order and first-order lights. The values are measured by Shimadzu's laser stray-light-measuring system. The latest development in the series of UV-VIS spectrophotometers is the UV-2700 which is a true double beam double monochromator system in a compact design for high-precision spectral analysis of a wide range of samples including organic and inorganic compounds, biological samples, optical materials and photovoltaics. The high performance optical system is designed with "LO-RAY-LIGH®" diffraction gratings, featuring highest efficiency and exceptionally low stray light. The spectrophotometer operates in the wavelength range of 185 to 900 nm and allows highly sophisticated applications such as direct measurement of high density samples up to 8 absorbance units without dilution. A typical example for high density measurements are $KMnO_4$ solutions in different concentrations which show an excellent linearity of up to 8 absorbance units. A variety of possible system configurations will be discussed on recent application examples and advantages of the new spectrophotometer series will be explained.

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Keywords: *Low Stray Light, Double beam Optics, Double Monochromator, Diffraction Gratings, UV-VIS spectrophotometer*

H25
FATTY ACIDS AND AMINO ACIDS PROFILE OF SOME VARIETIES OF LAGENARIA SICERARIA SEED

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Oil was extracted from the dehulled seeds of three Lagenaria siceraria varieties, derivatised and analysed for their fatty acids (FAs) composition using Gas Chromatography coupled with Mass Spectrometer (GC/MS). The defatted cake was analysed for amino acid composition using Technicon Multisample Amino Acid Analyser. The oil content of the seeds ranged between 46.5 and 48%. The major FAs in the oil were oleic (9.4–18.5%), stearic (7.7–8.9%), palmitic (13.6–15.3%) and linoleic acids (57.9–69.4%). The ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SAFAs), unsaturated fatty acids (USFAs) to SAFAs are 2:1 and 3:1 respectively, hence a good source of essential fatty acids (EFAs). Amino acid profile of the cake showed nine essential amino acids and eight non essential amino acids with lysine being the limiting amino acids.

Keywords: *fatty acids, amino acids, nutrition, seeds, oil*

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H26
PHYSICAL AND CHEMICAL CHARACTERISTICS OF HONEY BEES JATAÍ (TETRAGONISCA ANGUSTULA) OF LAJEADO CITY - RS - BRAZIL

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The large found by consumers for stingless bee honey has intensify the search in the characterization of these kind of product. In Brazil the searches has been directed in order to obtain a regulation standard for commercialization purposes. Honey bee jataí (angustula Tetragonisca) is popular due to its peculiar flavor very appreciate by consumers in several regions of Brasil. Research results showed that among species and native bee genera there is variation in the chemical composition of honey. From literature review we can observed that the lack of standardization in the collection is a factor that influences in the discrepancy found in the data for the same species. In this study were collected sample of Jatai honey bee' in the city of Lajeado, Rio Grande do Sul State, Brazil. The average values of some physico-chemical characteristics, considering six samples, were: moisture content 22.04%; reducing sugars 61.98%; sucrose apparent: 1.73%; ashes 0.48% and 36.70% total acidity. The data demonstrate a need for further study with regard to the honey harvesting time in order to avoid, in a standard regulation, for example, a sucrose content biggest in the product that those real at the end of ripening, that which could induce fraud.

Keywords: *stingless bee, honey, analysis, physico chemical, tetragonisca angustula*

H27
MICROSTRUCTURE ANALYSIS OF PROTEIN ISOLATES FROM LEGUME SEEDS

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Food protein microstructure is a very complex arrangement of protein molecules influenced by external and internal forces that are governed by the molecular properties of the protein. Visual examination by microscopy techniques such as light microscope, electron microscope, atomic force microscope and fluorescence microscope as well as investigation of protein molecular interactions are important in the analysis of protein microstructure. The objective of this work is to investigate the microstructure of isolated crystalline and amorphous protein structures from chickpea (CP), white kidney bean (WK) using NaOH extraction/ isoelectric precipitation (NaOH-protein isolates) and citric acid extraction/ cryoprecipitation (Cryo-protein isolates). The microstructure properties (particle size and morphology) of protein isolates stored at 40°C and 250°C for 0, 9, 18 and 27h were studied using dynamic light scattering. Particle size of NaOH-protein isolates was significantly ($p < 0.01$) h igher than particle size of cryo-protein isolates. Particle size (106.7 $x10^3$ nm) of NaOH-CPI measured at 0h was significantly ($p < 0.01$) higher than that of NaOH-WK: CPI and NaOH-WKI (71.5 $x10^3$ nm and 54.8 $x10^3$ nm, respectively). Storage of NaOH-protein isolates at 40°C for 9h increased ($p < 0.01$) particle size while storage at 25°C did not. Particle size of cryo-protein isolates increased ($p < 0.01$) gradually when stored at 40°C for 9, 18, and 27 h; light microscopic images of protein isolates supported these observations.

Keywords: *proteins, microstructur*

H28
DETERMINATION OF 13 DYES IN FOODS AND BEVARAGES BY HPLC–DAD

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The aim of the present work is to develop a multiresidual analytical method to determinate colorant residues in food according to annex II 1129/2011 of Regulation (EC) 1333/2008 that list the permitted dyes allowed in food products and their maximum residue limits. In order to detect permitted and not permitted dyes, a simple and suitable method has been developed for determination of 13 food dyes, azorubine (E 122), amaranth (E 123), cochineal red (E 124), red 2G (E 128), allura red ac (E 129), curcumin(E 100), tartrazine (E 102), quinoline yellow (E 104), sunset yellow (E 110), patent blue V (E 131), indigo carmine (E 132), brilliant blue FCF(E 133) and Orange II in confectionery products and beverages of wide consumption. Dyes from solid food matrices were extracted by a water-alcohol mixture, cleaned up on a polyamide SPE cartridge and eluted with a basic methanol solution. In order to perform the clean up activity, different solid phase materials were examined (Polymeric, C18 and Polyamide phase) and various elution solvent mixtures were also investigated to evaluate recoveries. Best results were obtained with polyamide SPE cartridge while the best elution solvent was NaOH 0.1% /methanol (1/1). Dyes from beverage matrices were diluted, filtrated and directly injected in HPLC without any further treatment. The HPLC–DAD method was developed on a Luna C8 column (150 mm, 4,6 mm, 3 µm) and validated according Regulation (EC) N° 882/2004. The method was applied in the range of 4–160 mg*Kg^{-1} (4–80 mg L^{-1} for drinks). Recoveries of dyes ranged between 65% and 110%, with an average RSD of 15%. In order to achieve a fast HPLC analysis the method was translate on a Poroshell 120 EC-C18 column (50 mm, 4.6 mm, 2.7 µm). The use of Poroshell is compliant with the separation of all colorants analysed in seven minutes and the validated method will be applied to a commercial samples selected on products of widest consumption. The present work, funded by the Italian Ministry of Health, was aimed to develop analytical methods fit for purpose in foodstuff intended for specific population such as infants and adolescents. As this field lacks of data, the main purpose of this study is to collect information through the analysis of the most used commercial samples, that will be useful for exposition studies.

Keywords: *dyes, foods, beverages, solide phase extraction, HPLC–DAD*

H29
SIMULTANEOUS DETERMINATION OF VITAMIN A (RETINOL) AND VITAMIN E (TOTAL TOCOPHEROLS) BY SIMPLIFIED LIQUID EXTRACTION AND REVERSE PHASE HPLC–FL

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The analysis of vitamins in foods is considered to be one of the most complex aspects of food chemistry. Particularly, the determination of oil soluble vitamins such as vitamin A and E is based on along and laborious extraction techniques. The majority of foods are fortified with α- tocopherol (vitamin E) and trans retinol (vitamin A). However other bio active forms can naturally occur (i.e. β-, γ- and δ- tocopherols). The determination of fortified levels can be relatively quick however determining the total amount of fat soluble vitamins normally requires a saponification step during which the fat bound vitamins are released into solution. As the released vitamins are not stable in an alkaline environment a series of solvent -solvent extractions are required to neutralise the solution prior to determination. The method developed is based on cold saponification followed by simplified liquid extraction (SLE). SLE was carried out using NOVUM™ extraction cartridges. The application is quicker than traditional solvent extraction and removes the risk of formation of emulsions and sample loss. The saponified extract is neutralised using strong acid and an aliquot is then extracted through NOVUM™ SLE. The solution is then concentrated and analysis is carried out using reverse phase HPLC with fluorescent detection. All four tocopherols, trans retinol and 13-cis retinol are then simultaneously determined. Simplified liquid extraction is significantly quicker than solvent extraction, allowing for a larger number of samples to be analysed. The short extraction time in combination with cold saponification is critical for vitamin analysis taking account of the sensitive nature of the compound. The method has been used in infant formula, milk powder, breakfast cereal and animal feed. A range of certified reference materials as well as 'off the shelf' food products have been used to prove equivalence with the traditional solvent extraction based methods and to validate this technique in line with ISO/IEC 17025 requirements.

Keywords: *vitamins, food, SLE, HPLC*

H30
MAXIMISING CHROMATOGRAPHIC SELECTIVITY FOR FOOD AND DRINK APPLICATIONS WITH RATIONAL UHPLC/HPLC STATIONARY PHASE DESIGN

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In this talk, we briefly review chromatographic selectivity and its importance for productive, rapid LC method development. Then, using a variety of chromatography data (including food and drink applications), we discuss stationary phase design principles. The resulting novel UHPLC/HPLC stationary phases show how it is possible to introduce functionality that enhances selectivity for analyte separation whilst maximising other desirable phase attributes such as phase stability, low bleed, reproducibility and mechanisms of interaction. Based upon the selectivity data, various 2 and 3 column LC method development platforms are defined and illustrated with food and drink applications for the novel stationary phase chemistries highlighted (ACE C18-AR, C18-PFP, SuperC18, C18-Amide and CN-ES). The talk finishes with LC column coupling experiments giving an effective LC column length of up to 450 cm to demonstrate very high peak capacities and high resolution s eparations for complex samples or natural product profiling.

Keywords: *HPLC/UHPLC, method development, selectivity, high resolution separations*

H31
THE STATE OF FOOD SAFETY IN PAKISTAN: OPPORTUNITIES & CHALLENGES

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The present review aims at highlighting the current status of food safety in Pakistan. It is an increasingly important public health issue, and a basic requirement for international trade. All consumers, whether domestic or in export markets have a right of safe food. Like other countries, Pakistan an agriculture country, is making efforts to meet its primary commitment of providing healthy food to her fast-growing population. Agriculture is a provincial subject, but provision of food and professional education are federal subjects. In 2011, after devolution of the Ministry of Food & Agriculture to provinces, a new division 'Food Security & Research' is established at federal level in view of exports and critical issues of ensuring food security. Pakistan does not have an integrated legal framework but has a set of four laws, which deals with various aspects of food safety. These laws have tremendous capacity to achieve at least minimum level of food saf ety. However, these laws remain very poorly enforced. Three of these laws directly focus issues related to food safety, while the fourth, the Pakistan Standards and Quality Control Authority Act, is indirectly relevant to food safety. Based on recommendations of the WHO, Codex Alimentarius, HACCP and FAO, provincial government of Punjab passed Food Authority Act 2011 while other provinces are in progress to develop Food regulations. In the agriculture sector, well established organizational set up and laboratories for monitoring biological, chemical, and physical contamination of foods exist at central as well as provincial levels. In Pakistan, frequent natural calamities and disasters make worse the challenges of food safety.

Keywords: *food safety, Pakistan, opportunities, challenges, legal aspects*

Acknowledgement: *PAEC*

H32
DEVELOPMENT OF EFFICIENT OXIDATION PROCEDURE FOR DYES ANALYSIS IN FISH TISSUES

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Malachite green (MG) and other triarylmethane dyes (crystal violet, brilliant green) are commonly use as a textile dyes. In addition, the dyes can be used in aquaculture industry due to their properties to prevent bacterial and fungal infections. In the present time it is known, that because of their cationic structure, dyes may deposit and persist in fish tissues and chitin of crustaceans and undergo metabolization in water organisms as well. Residual dye concentrations (predominantly in the form of metabolites) can be found in aquaculture products consequently to veterinary treatment or technogenic contamination. Metabolites of Malachite Green and Crystal violet – Leucomalachite Green (LMG) and Leucocrystal Violet (LCV) are reduced forms of parent compounds. MG and LMG have potential health and environmental hazards. In European Union (Council Directive 96/23/EC) and other countries, MG is not permitted for veterinary use due to its carcinogenic and muta genic properties. Regardless insufficient information about potential health and environmental hazards for crystal violet (CV), Brilliant Green (BG) and their metabolites, in 2010 EU established criteria for crystal violet residue concentration in shrimp and fish that do not allow more than 0.5 ppb (Decision 2008/630/EC and 2002/994/EC). According to Commission Decision 2004/25/EC Minimum Required Performance Limit (MRPL) was defined for sum of malachite green and leucomalachite green as a 2 µg/kg. The main problem of triarylmethane dye determination by mass spectrometry-based methods is weak analytical signal of the metabolites if using electrospray sources without heating, consequently their prior conversion (oxidation) to parent compounds is a critical step of sample preparation. Improving the efficiency of this process allows to reduce the Limit of Quantification. In our study we used extracts from homogenized tissue of Rainbow Trout, spiked with LMG, LMG and BG at 2.5 MRPL. DDQ and chloranil:acetonitrile mixture (in molar ratio 1:3) was used as oxidizing agent. Conversion of the metabolites was performed during evaporating stage under stream of nitrogen at 50°C. After conversion, extracts were purified with hexane and SPE. Detection was performed on Waters Acquity UPLC coupled to Bruker Maxis Q-TOF mass-spectrometer with Apollo electrospray source. The method included determination of the following compounds: sum of MG and LMG, sum of CV and LCV and Brilliant Gree n. The LC separation was carried on UPLC BEH C18 column (Waters), 1.7 µm, 1×50 mm. The detection was performed in MRM mode, total run time was 20 min. In 2012–2014 the method developed was successfully used in FAPAS proficiency testing scheme for sum of Malachite Green and Leucomalachite Green determination in fish muscle tissue. The main result of this work is significantly increased intensity of MG and CV analytical signals due to better metabolite conversion to parent forms (up to 80–90 %).

Keywords: *triarylmethane dyes, Rainbow Trout, malachite green, oxidation, mass spectrometry*

H33
VALIDATION OF 7 ARTIFICIAL SWEETENERS IN WATER WITH LC–HRMS USING DIRECT INJECTION

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After first introduction in the 1950´s, artificial sweeteners have recently been found in surface and tap water in different regions. Mainly unmetabolized by human consumption and hardly eliminated by wastewater treatment plants, artificial sweeteners have become ubiquitous throughout the aquatic environment. Due to its persistence against microbial degradation, Acesulfame is often used as marker for contamination by municipal waste water. In this way even wastewater-derived proportions of less than 1‰ of a water resource can be detected. In order to determine seven artificial sweeteners in table water and according raw water, an analytical method without time consuming sample pre-treatment such as SPE, which may cause analytical errors (analyte loss and sample contamination), was developed. Related to the German Orientation Levels for pesticide residues and pharmaceuticals in table water, an analytical method with a Reporting Level of 50 ng/L according to SANCO 12571/2013 using 100 µl direct injection into UPLC–Orbitrap–HRMS was validated. Pure standards of Acesulfame, Aspartame, Neohesperidine, Cyclamate, Saccharin, Neotame and Sucralose were dissolved, diluted and injected into the UPLC–HRMS. The exact mass of at least one fragment-ion according to the exact mass of the molecule-ion and the retention-time was identified. Measurement-mode was data dependent MS2 with a full scan resolution of 70.000 and a resolution of 35.000 for fragment-ions. 100 µL degased and filtered table water were injected. Spiked table water at 50 ng/L and 500 ng/L showed recovery rates between 86 and 115%. The variation coefficient at 50 ng/L was below 5.3% for all analytes except Saccharin. For Saccharin a Reporting Level of 500 ng/L with a variation coefficient of 4.4% was derived. Calibration showed linear regression over at least 3 orders of magnitude starting with the lowest acceptable calibration level of 10 ng/L (for Saccharin 250 ng/L). 15 samples of table water and according raw water were analyzed with the described method. In 2 samples artificial sweeteners (Acesulfame, Cyclamate) were found with concentrations up to 227 ng/L.

Keywords: *artificial sweetener, LC-HRMS, Orbitrap, water*

H34
ASSESSMENT OF MINERAL COMPOSITION INCLUDING TOXIC ELEMENTS OF PROPOLIS BASED ON NEAR INFRARED SPECTROSCOPY

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The potential of near infrared spectroscopy (NIR) with remote reflectance fiber-optic probe for determining the mineral composition of propolis was evaluated. This technology allows direct measurements without prior sample treatment. 91 samples of propolis were collected in Chile (Bio-Bio region) and Spain (Castilla-León and Galicia regions). The minerals measured were aluminum, calcium, iron, potassium, magnesium, phosphorus, and some potentially toxic trace elements such as zinc, chromium, nickel, copper and lead. The regression method modified partial least squares (MPLS) was used to develop the NIR calibration model. The results demonstrated that the capacity for prediction can be considered excellent, for wide ranges of concentration for potassium, phosphorus and zinc; and acceptable for aluminum, calcium, magnesium, iron and lead. This indicated that NIR method is comparable to chemical methods. The method is of interest in the rapid prediction of potentially toxic elements in propolis in the margins (Zn: 0–144 ppm; Cr: 0.5–5.3 ppm; Ni: 0–4.2 ppm; Cu: 0–5.8 ppm; Pb (0–14.6 ppm) before consumption.

Keywords: *propolis, mineral composition, toxic elements, NIR, cross-validation*

Acknowledgement: *This study was made possible by funds from Project 18KBCN/463AC01 of University of Salamanca. The authors especially to thank the cooperation of beekeepers in this study*

H35
CHEMICAL COMPOSITION DETERMINATION IN MEALS READY TO EAT (MRE) BY NEAR INFRARED SPECTROSCOPY

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Due to industrialization and life style nowadays the feeding behavior has considerably changed, especially in developed countries and people have less time and turn more and more to MRE. The definition of a MRE is a dish that has been transformed, packed, labelled and can eventually be warm up. As most of these MRE are not always nutritionally well balanced, the consumers are facing problems of obesity which is a major risk factor for many illnesses as diabetes, cancers and cardiovascular diseases. Therefore a correct labelling of these products with clear and accurate nutritional information is the best way to inform effectively the consumers. Anyway it is now a legal requirement and the EU regulation No 1169/2011 forces the producers to label correctly all their products. In this context it is obvious that a rapid, cost effective and accurate technique analysis as Near Infrared (NIR) spectroscopy could be a useful tool to quantify the main quality parameters of the MRE. The objective of this study is, then, to develop NIR calibrations to quantify the chemical composition of meals ready to eat (MRE). 150 samples of MRE including a large variety of products as lasagna, pizza, pie, hamburger, Asiatic dishes, dishes based on meat or fish mixed with vegetables have been analyzed by wet chemistry to quantify protein, fat and dry matter. The energy value was determined by a calculation based on the concentration of the previous properties. Given the different kind of products, the nutritive composition of MRE can vary in huge proportion. All these samples have been mixed in a Grindomix to obtain a kind of puree which was scanned 5 times on a Foss NIRS XDS spectrometer at room temperature. The dataset was divided into a calibration set (120 samples) and a validation set (30 samples) and PLS calibrations were developed using the range 1100–2500 nm. The validation set was selected to cover the range for each parameter. As RPD values obtained for the validation are higher than 3, this suggest that NIR can effectively be a useful technic for the producer to label properly their products but also for the control instance to check the information showed on the labels. This study has shown that it is possible to use universal calibrations regardless of the type of MRE to quantify the main properties, including the energy values.

Keywords: *meal ready to eat (MRE), NIRS, protein, fat, energy*

H36
SCREENING OF HERBAL MEDICINES AND DIETARY SUPPLEMENTS FOR SYNTHETIC COMPONENTS

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One of the most actual and difficult problems of herbal remedy analysis is identifying unlabeled synthetic components. It is known that many herbal products and health supplements marketed for sexual performance enhancement are falsified by adding special synthetic compounds. According to literature, more than 50 unapproved analogues of prescription PDE-5 inhibitors are used as adulterants [1]. Disseminators of illegal products for sexual performance enhancement are investing time and resources to synthesize exotic analogues and devise novel means for adulteration in order to avoid identification of counterfeited products by standard screening protocols. In this work a methodology, based on the combination of DART-MS and NMR 1H spectroscopy, was found to be highly promising for fast screening of such samples for synthetic additives. 98 commercial samples were analyzed using the proposed methodology. 73 of them contained illicit synthetic compounds. The most common adulterant (probably due to the easiest synthetic pathway) was tadalafil, which was found in 54 samples. Spectral information, especially molecular weight from DART mass spectra and chemical shifts from NMR spectra, confirmed a presence of aminotadalafil, sildenafil, chloropretadalafil, nortadalafil and thiohomosildenafil in different samples. Analyzing NMR spectra allows to quantify such components without a need in respective standards.

[1] D.N. Patel et al. Screening of synthetic PDE-5 inhibitors and their analogues as adulterants: Analytical techniques and challenges // J. Pharm. Biomed. Anal. 87 (2014) 176-190.

Keywords: *herbal medicines, dietary supplements, PDE-5 inhibitors, DART-MS, NMR spectroscopy*

H37
ADULTERATION OF DIETARY SUPPLEMENTS WITH SYNTHETIC PHOSPHODIESTERASE TYPE 5 INHIBITORS IN RUSSIAN FEDERATION

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Adulteration of dietary supplements, claiming to enhance sexual potency, with deliberately added active pharmacological ingredients is widespread all over the world. The aim of this study was to determine whether dietary supplements on the Russian market contain phosphodiesterase type 5 (PDE-5) inhibitors, such as sildenafil and tadalafil. Fourteen samples of 6 most popular products were analyzed with HPLC–DAD–MS/MS. Manufacture was claimed as United States (n=2), Malaysia (n=2), and Russian Federation (n=2). PDE5-inhibitors were detected in 11 (78.6%) samples, including tadalafil (n=10) and tadalafil with sildenafil blend (n=1). Five samples of 2 supplements contained tadalafil only in caplsule shell but not in the capsule content. There were pronounced heterogeneity of inhibitors contents between samples within individual products with variation from null to 31.1 mg per capsule probably due to lack of quality control during manufacture. The amounts of tadalafil were higher than the maximum recommended dose in 4 (36.4%) of dietary supplements tainted with this drug. As food products dietary supplements are not subjected to postregistration analysis. That affords unscrupulous manufacturers to put on the market adulterated products those, in fact, are misbranded drugs. Refined federal control measures and effective, cheap and simple analytical techniques are required to protect the health of male population in Russian Federation.

Keywords: *dietary supplements, HPLC–DAD–MS/MS, tadalafil, sildenafil*

H38
COMPARATIVE STUDY OF DIETARY FIBRE FRACTIONS EVALUATED BY THE NEW INTEGRATED METHOD AND THE TRADITIONAL ANALYTICAL PROCEDURE FROM NON-CONVENTIONAL FRUIT BY-PRODUCTS

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Dietary fibre (DF) has recently attracted attention due to its well-known physiological and functional properties. Numerous sources of DF have been studied; nevertheless, the use of non-conventional matrices, such as fruits and vegetable by-products, obtained by food processing industry has gained interest. The accurate determination of total (TDF), soluble (SDF) and insoluble (IDF) dietary fibre is relevant in terms of food analysis research and nutritional labelling. The AOAC methods traditionally used for this purposed (991.43 and 985.25) have important differences (enzymes, digestion times and temperatures, filtration setup, HPLC quantification, among others) from the new integrated total DF assay procedure (AOAC 2011.25/AACC 32-50.01). These differences permit the obtaining of DF fractions including IDF and two fractions of SDF: high and low molecular weight (HMWSDF and LMWSDF, respectively), including resistant starch and non-digestible oligosaccharide s. These fractions coincide with the recently proposed CODEX definition of DF. This methodology has been mostly performed for cereals, cereal based products or starchy foods; however, its application in non-conventional sources, such as fruits and fruit by-products, need to be further studied. The aim of this study was to compare the AOAC traditional method (TM) with the AOAC new integrated method (NIM), on the evaluation of SDF, IDF and TDF content from orange, mango and prickly pear peels. For orange and prickly pear peels, the SDF content was 1.1 times greater in the TM than in the NIM for both fruits. The IDF fraction for orange was, as well, 1.1 times larger in the traditional procedure; however, this content was consistent in both methodologies for prickly pear. TDF content measured by the NIM was about 7.0% and 2.0% lower for orange and prickly pear, respectively, suggesting an slightly overestimation of SDF in the TM. In mango, on the other hand, the content of SDF evaluated by the conventional assay was lower (about 7.0%), which is consistent to previous reported evaluations on cereal based foods. Also, a considerable minor IDF content for the TM was observed (about 25.0%). Moreover, TDF in mango evaluated by the NIM was about 16.0% larger, suggesting that this new approach might be capable to quantify additional IDF, depending on the source specific characteristics. Finally this research showed inconsistences on the DF quantification between both methodologies, which not only depend on the SDF:IDF ratio, but also on the evaluated fruit. Comparisons between the mentioned methodologies utilising more matrixes are needed in order to select the accurate methodology for each food, and/or to improve the existent analytical procedures.

Keywords: *dietary fibre, fruit by-products, Insoluble dietary fibre (IDF), SOluble dietary fibre (SDF)*

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H39
APPLICATION OF GAS CHROMATOGRAPHY – VACUUM ULTRAVIOLET ABSORPTION DETECTION FOR THE ANALYSIS OF FATTY ACID METHYL ESTERS

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Fatty acids and their corresponding methyl esters (FAMEs) are important analytes for consideration in terms of food science, nutrition, and bio-based fuels. Typically, these are characterized by gas chromatography – mass spectrometry (GC–MS), but the complexity of the system, as well as many closely related isomers and isobars, can make complete speciation difficult. We have applied a new vacuum ultraviolet absorption detector for GC (GC–VUV) to demonstrate its superior capability for FAME characterization. GC–VUV measures the absorption of eluting compounds in the 115–240 nm range where all chemical species absorb. Each FAME and class of fame has unique absorption features that enable both qualitative and quantitative analysis. The differentiation of FAMEs is demonstrated with standard mixtures, as well as mixtures of FAMEs prepared from various food oils. GC–VUV is shown to be extremely well adept at characterizing FAME compositions from real oil samples without significant interferences.

Keywords: *fatty acids, VUV, VACUUM ULTRAVIOLET, FAME, GC-VUV*

H40
STUDYING THE BACTERIAL DIVERSITY OF TABLE EGGS USING DIRECT SEQUENCING OF THE 16S RRNA GENE

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The aim of this study is to identify the bacterial diversity of table eggs using a direct sequencing approach for identifying viable but none culturable (VBNC) bacteria. Direct isolation of bacterial genomic DNA from eggshell rinse and egg content homogenate were successfully developed. The extracted DNA fragments were amplified by PCR using Easy-A high-fidelity enzyme, then ligated with a 5-T overhang vector, and cloned in Escherichia coli. Purified clones were obtained by growing them on LB agar containing a selective antibiotic. Plasmids were extracted, and the presence of a cloned gene was confirmed by digestion with restriction enzyme EcoRI. Preliminary results of sequencing 23 clones have revealed that 48% of the sequences are related to the 16S rDNA sequence of Psychrobacter spp.

Keywords: *DNA, 16SrRNA, cloning, psychrobacter, egg*

Acknowledgement: *This work is supported by Saudi municipality. Many thanks go to my family for their support and encouragement.*

H41
ASSESSMENT OF PLANT OILS OXIDATION STABILITY EMPLOYING ALTERNATIVE ANALYTICAL STRATEGIES

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Cold-pressed oils are very popular among consumes because of their nutritional value and unique flavour. Main conditions during technology process, specifically low temperature, allow to retaining attractive features of these products. With regards to their higher cost, adulteration /mislabeling of cold-pressed oils become an issue of serious concern.

In the first part of our study, we focused on the assessment of oxidative stability that is one of the key quality parameters closely associated with sensoric properties does product shelf-life.

12 various cold-pressed oils (including those prepared from roasted seeds) were oxidized (4,4 g of oil) in a closed 44 ml vessel at 60°C. Samples were taken for analysis after 2, 4, 7 and 10 days. For determination of oxidation extent and measurement of components including this process, following tests were performed: (i) determination of peroxide value; (ii) determination of antioxidant activity using radical scavenging method (DPPH) and (iii) spectrophotometric measurement of total phenolics content: (iv) profiling of oil volatiles by solid phase microextraction coupled with gas chromatography - hight resolution mass spectrometry (SPME–GC–HRMS).

The study documents large difference among oxidation stability of oils showing white sesame oil as the most stable. The volatile profiles were assessed against other parameters measured. As far as the number of GC peaks and their intensity detected during oxidation experiment is considered as parameter, then the best correlation was found with peroxide value. The statistical processing of volatile profiles using Principal Component Analysis (PCA) showed clear separation of samples according to their (i) biological origin (the most pronounced separation of flaxseed oil), (ii) state of oxidation. Methylpyrazine and 2,5-dimethylpyrazine were identified as markers of oil from roasted seeds.

Keywords: *cold-pressed oils, oils of plant, oxidative stability*

H42
ORGANOLEPTIC INDEXES OF COMBINED MEAT CUT HALF FINISHED GOODS FROM FERMENTED RAW-MATERIAL

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An investigation of new resources providing optimal correlations of chemical food components for an organism is a scientific base of a modern strategy of food production. The main moment of this problem is the search of new sources of proteins and vitamins. Under conditions of the animal proteins lack in people's food an urgent problem is more absolute use of available resources. An enzymatic processing is used with the purpose of properties improvement of meat raw materials of little value in respect of food. The enzymatic processing makes it possible to increase a biological value and improve organoleptic indexes of low-grade meat raw material. Sources of food protein on basis of plants have a high biological value due to the content protein substances concerning a good assimilability and nutritional property and also a low content of fat. It occurs wide opportunities for a purposeful use of vegetable proteins as additives when producing meat foods and as the main component of combined products. In the research the results of the appraisal of organoleptic qualities of combined meat half finished goods produced from low-grade meat raw material – grade B beef that is exposed to a preliminary ferment processing. Vegetable oil and oatmeal are placed to the formulation of half finished goods for increasing food value and attaching product dietary properties. All the above components are placed when making minced meat of half finished goods in a preliminary prepared protein-fatty emulsion. The results of the organoleptic appraisal show that a homogeneous consistence is ensured and the structure of minced meat is improved when half finished goods of fermented meat raw material is placed to the minced meat, because oatmeal together with the other components of the emulsion makes dispersions of low viscosity, acting like a linking agent between oil and moisture. Prototypes of half finished goods have a nice taste and smell. On the section half finished goods have a state of a well-mixed minced meat. The prototypes have more dense and elastic consistency and also are more succulent and less crumbled as compared with control samples. The derived results show that bringing in minced half finished goods protein-fatty emulsion consisting from fermented meat raw material, oatmeal and vegetable oil conduces to the improvement of organoleptic indexes.

Keywords: *organoleptic indexes, fermented raw-material, protein-fatty emulsion*

H43
SENSORY EVALUATION OF OLIVE PASTES PACKED IN TUBES OVER STORAGE

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Table olive is a typical Portuguese food and an important ingredient in Mediterranean diet. Olive production has an increasing economic importance and a valuable tradition in Portugal. Despite the increased olive production, table olive industries sometimes find it difficult to sell small caliber fruits, as well as to find applications for fruits that are physically damaged or defective. It is therefore necessary to look for alternatives to reduce or minimize economic losses and at the same time to add value to this important agricultural product. One alternative that was explored in this work, is the development of olive pastes. The study had the collaboration of meia.dúzia®, a company specialized in the production of fruit jams packed in tubes, whose goal is to achieve sustainability through the constant introduction of new products in the market. Packaging in tubes has the advantage that light does not interact with the product. Also, after opening the exposure to air is reduced in comparison to other types of packages, minimizing oxidation processes and maintaining the original colors. The raw materials used in this study were green and black olives (Olea europaea L.) in brine. Green olives were washed in water to remove the brine and were subsequently crushed in a blade mill until a homogeneous paste was obtained. This initial olive paste was divided into 2 parts, one condimented with garlic, the other with garlic and rosemary. A black olive paste was obtained following the same procedure, and divided in two parts, one condimented with honey and the other with rosemary. After mixing all ingredients, the pastes were packed in tubes, sealed and pasteurized at 85ºC for 10 min. Sensory quantitative descriptive analysis was performed with 8 semi-trained panelists and acceptability tests of the four pasteurized olive pastes were carried out until 120 storage days. For each sensory parameter, student t tests were used to detect significant differences between samples (attributes). Data analyses were complemented with principal component (PCA), looking for main v ariation patterns. Green olive pastes (honey and honey/rosemary) and black olive pastes (garlic and rosemary) with 15 days of storage presented more intense olive and spicy aroma and flavor. After the 90th day of storage, both black and green olive pastes had higher acidity and less brightness, salty taste and spreadability than in the early storage days. Sensory analysis showed that panelists detected some defects in smell and taste attributes in olive pastes, mainly after the 30th storage day. Significant statistical differences ($p < 0.05$) were detected between black and green olive pastes in some sensory parameters, over storage time. Principal component analysis representing 59% of initial information showed that green and black olive pastes with 6 storage days have more olive flavor and aroma and are sweeter and more intense in honey aroma.

Keywords: *table green and black olive, olive pastes, Sensory quantitative descriptive analysis, Principal component analysis, shelf life*

H44
SENSORY ACCEPTANCE OF ARTISANAL MEAD DRY TYPE

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This work aimed to evaluate the sensory characteristics of three dry type meads with different maturation times: six, twelve and twenty-four months. The sensory analysis was done by 80 individuals that had interest. To participants was applied a questionnaire containing socio-economic questions. After, they evaluated the product using a structured hedonic scale of nine points to measure both the degree of general acceptability and specific attributes. The samples of six and twelve months were considered most accepted among participants and the twenty-four months sample considered the less accepted. In assessing the acceptability for the different attributes (color, aroma, body and texture, alcohol content, acidity and flavor) it was observed that to mead of twenty four months were attributed the worst averages, had difference statistically in the media value to a level of 1% (Tukey test). The results obtained showed us that the maturation time had influence on the characteristics of the final product and the mead of twenty four months was what gained less acceptance for both the general characteristics and their specific attributes. We can conclude that the aging process had influence in the characteristics of the product here evaluated indicating that these artisanal meads can be sold until 12 months after their production. In order to maintain their acceptance and flavor it's necessary control of environmental conditions during the aging time.

Keywords: *mead, sensory, artisanal, sensory characteristics, acceptance*

H45
SENSORY QUALITY CONTROL OF READY-TO-USE VEGETABLES IN MAP

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A study of the shelf life of cabbages, carrots, green beans and yellow, red and green peppers, packed in modified atmosphere (MA), all in the form of fresh cuts and precooked samples was carried out. Fresh cuts were stored over 17 days with 10%O_2/45%CO_2, and pre-cooked samples over 28 days with 0%O_2/40%CO_2, all at 5±1°C. In order to conduct this study to evaluate the shelf life of fresh cut and pre-cooked vegetables it was necessary to use sensory judges no acquainted with this type of products. A list with 17 attributes using 13 point scales was agreed. To overcome the problem of lack of training, scales were anchored at the midpoint with "fresh". During tasting sessions, 15 judges had in their presence a sample of the fresh product (standard), whose scoring corresponded to the midpoint of each attribute scale. It was assumed that samples would lose or gain points in quality attributes during storage, when compared to the standard. Therefore judges' quantifications reflected distances between stored and fresh samples. The same strategy was followed for pre-cooked vegetables, using fresh vegetables cooked immediately before analysis as standards. Products were analysed at regular intervals along storage time for changes in organoleptic properties, as well as in acidity, total phenols, anthocyanins and flavonoids, antioxidant activity and instrumental texture and colour. The utilization of a standard together with a normal QDA proved to be an improved and very reliable technique, as demonstrated by repeatability and reproducibility studies based on coefficients of variation and ANOVA/MANOVA. Sensory attributes were very well correlated with the corresponding chemical and physical parameters, as demonstrated by automatic predictive biplots applied to principal components and canonical correlation analyses. Depending on the vegetable, some changes were observed, but not on a concerning level, since judges did not perceive loss of quality except for samples at the end of the study time, results that were supported by chemical and physical analyses.

Keywords: *shelf life, modified atmosphere pakaging, ready-to-use vegetables, sensory analysis, quantitative descritive analysis*

METALS AND METALLOIDS

(I1 – I15)

I1
ANALYTICAL AND CHEMOMETRICAL EVALUATION OF PU-ERH AND FRUIT TEA QUALITY IN VIEW OF THEIR MINERAL COMPOSITION

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Among the most reckoned beverages around the world is tea, which has a significant positive impact on human health. It contains many bioactive substances such as polyphenols, flavonoids, amino acids, proteins, enzymes, vitamins, carbohydrates and trace elements. Macro- and microelements play a vital role in the metabolic processes and general well-being of human body. However, their concentration should be also monitored, especially in view of permissible limits for growth and good health. The aim of the study was to evaluate Pu-erh and fruit teas quality based on their mineral composition. Macro-, microelements and toxic metals levels (Mg, Ca, K, Na, Mn, Cu, Fe, Zn, Cr, Ni, Co, Cd and Pb) in red (Pu-erh) and fruit tea leaves and their infusions were determined by F-AAS (Flame Atomic Absorption Spectrometry) method. Phosphorus was quantified in the form of phosphomolybdate by spectrophotometric method. Based on the mineral composition it was possible to estimate leaching percentage and assess the risk of exceeding Provisional Tolerable Weekly Intake (PTWI) for heavy metals (Pb, Cd) through daily tea consumption. There was also calculated realisation of the Recommended Daily Intake (RDA) for the analysed bioelements according to the latest available standards. Reliability of the method was checked using three certified reference materials and the results of analysis were highly satisfying. Based on the obtained data it can be concluded that the analyzed teas were characterized by varied metals contents. The greatest differences in Pu-erh tea were noted for K, P, Ca and Mg (2665, 1430, 821 and 322 mg/100 g, respectively). Fruit tea and black tea with fruits were characterized by the highest content of Mn (22-76 mg/100 g) and Fe (22–34 mg/100 g). Due to the application of correlation analysis, significant interdependences between concentrations of P, Zn, K, Ni, Cr and Pb in Pu-erh tea and between Na, Fe, Mn, Ni and Cr in fruit teas were obtained. Furthermore, ANOVA Kruskal-Wallis test results have related differences in Pu-erh tea quality as well as technological processing of fruit tea to their mineral composition. In order to characterize tea elemental content, chemometric techniques such as FA (Factor Analysis) and CA (Cluster Analysis) were used. Their application allowed on differentiation of samples in view of the type of fermentation, technological processing and overall quality.

Keywords: *Pu-erh tea, fruit tea, bioelements, F-AAS*

I2
BIOACCESSIBILITY OF ESSENTIAL AND NON-ESSENTIAL ELEMENTS IN CHOCOLATE BARS

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Chocolate bars are the most consumed cocoa-derived product worldwide, mainly due to their pleasant palatability. Besides that, among the beneficial features of chocolate products are their antioxidant activity, high carbohydrate content and presence of some essential metallic elements [1]. On the other hand, some potentially toxic elements at trace levels were also found in this type of food [2,3]. The determination of the total contents in foodstuffs brings the essential information to evaluate the presence of metallic elements, but a more adequate nutritional and risk assessment evaluation can be done by evaluating their oral bioaccessibilities in order to estimate the fraction of the ingested compound that is released into the human gastrointestinal tract and is available for intestinal absorption. Despite of the large consumption of chocolate bars, there is no available information about the metallic elements bioaccessibility in this matrix. Taking this into account, the main objective of this work is to evaluate the presence of metallic elements in dark, milk and white chocolate bars by estimating their bioaccessibilities, including essential (Cu, Fe, Mn, Zn, Cr and Se) and non-essential elements (Al, Ba, Sr, As, Co, Ni and V) by applying an in vitro digestion method that simulates the gastric and intestinal human digestion. The analytical determinations were made by Inductively Coupled Plasma Mass Spectrometry (ICP–MS) after microwave-assisted sample treatment. The bioaccessibility fractions were determined in five replicates for each type of chocolate and the results were in the range of 45–63% for Co, 26–89% for Cu, 4–67% for Fe, 29–89% for Mn, 8–89% for Zn, 28–36% for Cr, 9–89% for Se, 3–5% for Al, 13–55% for Ba, 25–86% for Sr, 42–62% for As, 50–63% for Ni and 76–89% for V. The highest total concentrations of all elements were found in dark chocolates and the lowest in white chocolate. However, in white chocolate the e lements exhibited bioaccessible fractions significantly higher (55–89%) when compared to milk (5–63%) and dark (3–50%) chocolates, suggesting that the bioaccessibility should be consider together with the total concentrations for assessing the presence of elements in foodstuffs.

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Keywords: *bioaccessibility, chocolate bars, essential and non-essential elements, essential and non-essential elements, in vitro digestion methods, ICP–MS*

Acknowledgement: *Fapesp (process 2013/26855-2), CNPq, INCTAA, Agilent Technologies*

I3
CHARACTERIZATION AND QUANTITATIVE ANALYSIS OF HEAVY METALS IN WINE USING UV-VIS AND ICP-OES SPECTROSCOPY

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Wine is one of the oldest cultural products in human history. Wines have been cultivated for over 8000 years. The oldest known archaeological evidence of winemaking is an 8000-year old wine- and fruit press found near Damascus.
Winemaking is a rather simple process: freshly harvested grapes are crushed and the resulting juice (must) is collected. The must contains fermentable sugars and natural yeasts, which, either by themselves or with the help of additional yeast cultures start the fermentation process in which mainly ethyl alcohol and carbon dioxide are formed. The latter is a gas and escapes from the must. The fermentation process comes to a halt when all of the sugars are fermented or the alcohol concentration becomes too high and kills off the yeasts. At this point the must has turned into wine.
In order to guarantee a certain level of quality, standards are fixed in the national wine regulations such as the German "Weinverordnung" [1] from 27th April 2015, which includes the classification of wines from different locations but also the production process, alcohol concentrations and the maximum allowable concentrations of elements.
A meticulous quality control procedure is essential, and during each stage of the production process spectroscopic methods such as AAS-, ICP-, FTIR-, and UV-VIS spectroscopy are applied for quality assurance or for product characterisation. The colour of the red wine is an important factor to understand the quality of the raw material but also the treatment and the storage. Typical measurements have to be performed at 520 nm for example using a UV-VIS spectrophotometer [2].
For the quantitative determination of essential elements such as potassium, sodium, calcium and magnesium, as well as heavy metals such as lead, arsenic, and cadmium, the ICP spectrometry is the method of choice, since a simultaneous instrument, such as the Shimadzu ICPE-9820 allows a fast and precise analysis.
In order to round off the analytical procedure for detecting undesired substances such as 2,4,6-Trichloroanisole in wine headspace-trap GC/MS has been used offering the separating power of gas chromatography (GC) with the detection power of mass spectrometry (MS).

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Keywords: *wine analysis, ICP–OES spectrometry, UV-VIS spectrometry, hHeavy metals, headspace GC-MS*

I4
INVESTIGATION OF THE CR(VI) BEHAVIOR IN FOODS BY HPLC–ICP MS

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Chromium is an element that exists under two oxidation states, Cr(III) and Cr(VI). Whereas Cr(III) is essential to life, Cr(VI) is considered carcinogenic. Cr(VI) speciation is now performed in routine for environmental samples (water, soils…) by HPLC–ICPMS. However it is much more difficult to achieve in foods due to extraction problems, carbon interferences and the lack of knowledge on the behavior of the chromium linked with the matrix. The objective of our work was therefore twofold:
- to investigate the reactions occurring between Cr(VI) and the food matrices
- to develop a specific and sensitive analytical method for Cr(VI) quantification in such samples.
Different kinds of samples have been investigated like milk and dairy products, fruits and vegetables, fish, meat, etc… The presentation will display firstly the interactions between Cr(VI) and the different matrices investigated (by size-exclusion HPLC–ICPMS). The method developed for the specific Cr(VI) measurement by ion-exchange HPLC–ICPMS will then be detailed in terms of analytical development, validation criteria obtained in the absence of Certified Reference Material and its application for the determination of Cr(VI) in a wide range of products from a local supermarket. Finally, the method developed has been applied to study the stability of Cr(VI) in milk over storage and cooking.

Keywords: *hexavalent chromium, HPLC–ICPMS*

I5
METHYLMERCURY DETERMINATION WITH SPECIFIC EXTRACTION AND ADVANCED MERCURY ANALYZER IN MARINE BIOTA TISSUES – COMPARISON WITH ISOTOPIC DILUTION GC–ICPMS

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Mercury is a persistent pollutant, particularly in the form of methylmercury (MeHg), a potent neurotoxin present in marine environments. MeHg is bioaccumulated in tissues from marine living organisms where concentrations can be very high and its determination needs to be performed now on a routine basis. The reference method for Hg speciation in food from marine origin is Gas Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry (GC–ICP MS) after alkaline extraction, derivatization and quantification using species isotope dilution quantification. This method is currently subject to standardization by the European Standardization Committee (CEN). In spite of its good sensitivity and accuracy, its implementation is long and complex and requires an estimation of the total Hg concentration. Therefore, an alternative technique has been optimized in the aim to propose a simple, fast and cost-effective method for methylmercury determination. This method is based on a specific methylmercury extraction with an organic solvent (toluene), its recovery in an aqueous solution which is directly analyzed using an advanced Hg analyzer (AMA 254). After optimization, performances obtained in terms of limits of quantification, repeatability and reproducibility and accuracy are comparable to GC–ICPMS. The developped method was then applied to oyster and mussel samples collected in Southern Europe in the frame of the Interreg ORQUE SUDOE project. Methylmercury concentrations obtained are in very good agreement with results from ID GC–ICPMS and were also validated by an inter-laboratory comparison exercise organized in this project.

Keywords: *methylmercury, AMA, GC–ICP MS, marine biota*

I6
DETERMINATION OF ESSENTIAL AND TOXIC ELEMENT IN SOUTH AND CENTRAL ITALIAN HONEY SAMPLES BY ICP-MS

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According to the definition set by the European Union Council Directive 2001/110/EC, "honey is the natural sweet substance produced by honey bees, Apis mellifera, from the nectar of plants (blossoms) or from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature." Honeybees are continuously exposed to potential pollutants present in widespread foraging areas, and the influence of industrial pollution on bee health has been widely documented (Leita, et al. 1996). This makes honey a matter of interest in food safety studies, particularly bearing in mind that the majority of consumers are children. Honey is composed mainly from carbohydrates (75%), lesser amounts of water and a great number of minor components. Minor constituents include essential and toxic metals and unidentified substances. Regarding metal contents, previous investigations have shown that their presence depend mainly on the botanical origin of honey (Gonzalez-Miret et al. 2005). In order to assure food safety, honey should have a low content of undesirable contaminants. The aims of this study were the identification and quantification of toxic and essential elements in 70 honey samples collected from 10 different provinces of Central and South Italy. The content level of 24 elements (Hg, Tl, Pb, Cd, Cr, U, Ti, Ba, Sb, Al, As, V, Ge, Sn, Be, Sr, Ca, Fe, Mn, Co, Zn, Cu, Se, Mo) were determined using Microwave Assisted Extraction coupled with Inductively Coupled Plasma Mass Spectrometry (MAE-ICP-MS). Results demonstrated that although samples are not completely contaminant free, heavy metal intake from honey is well below the recommended dose. Furthermore, chemometric methods highlighted differences among honeys depending from their geographical and botanical origins.

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Keywords: *honey, toxic elements, essential elements, ICP–MS*

I7
DETERMINATION OF TOTAL AND INORGANIC ARSENIC LEVELS IN RICE USING LC–ICP–MS IN GYEONGNAM PROVINCE OF KOREA

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The arsenic, has long been recognized as a poison, was composed of both organic and inorganic arsenic forms. Inorganic arsenic compounds includes trivalent As(III), pentavalent As(V), monomethyl arsenate(MMA), dimethyl arsenate(DMA) and etc.Especially, trivalent As(III) and pentavalent As(V) of inorganic arsenic species are more toxi- city than others.This survey estimated the total arsenic(t-As) and inorganic arsenic (i-As), especially trivalent As(III) and pentavalent As(V), of rice in Gyeongnam province of Korea. The arsenic species levels of samples were determined using micro-wave extraction and malonic acid method. Arsenic separation was carried out via LC with RP-100 column with ICP–MS detection (LC–ICP–MS). The detection limits (LOD) and quantification limit (LOQ) for arsenic species (t-As, i-As) were established. The t-As and i-As levels in 48 brown rices ranged between 69.612 µg/kg and 318.764 µg/kg(mean 145.950 µg/kg), between 42.044 ug/kg and 186.588 µg/kg (mean; 89.322 µg/kg). The t-As and i-As levels in 48 polished rices ranged between 31.467 µg/kg and 156.110 µg/kg (mean; 76.052 µg/kg), between 24.203 µg/kg and 94.837 µg/kg (mean; 53.414 µg/kg). Further, based on the Korean public nutrition report 2005, these levels are calculated at % Provisional Tolerable Weekly Intake (PTWI) of this study which has been established by FAO/WHO. Therefore, the levels presented here are presumed to be adequately safe and our results can be utilized as a reference to establish specific standards for rice in Korea.

Keywords: *rice, arsenic, heavy metal*

I8
BIOAVAILABILITY OF CADMIUM FROM LINSEED AND COCOA

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The exposure of the European population to cadmium from food is high compared with the tolerable weekly intake of 2.5 µg/kg bodyweight set by EFSA in 2009. Only few studies on the bioavailability of cadmium from different food sources has been performed but this information is very important for the food authorities in order to give correct advises to the population. The aim of this study was to investigate the bioavailability of cadmium from whole linseed, crushed linseed, cocoa and cadmium chloride in rats. An experiment where 40 rats were divided into 4 groups and a control group and dosed with whole linseed, crushed linseed, cocoa and cadmium chloride for 3 weeks was performed. Linseed or cocoa made up 10% of the feed (by weight) and was added as a replacement for carbohydrate source. The rats were dosed for 3 weeks and the cadmium content in the rats' kidneys was measured by ICP–MS as a biomarker for the exposure during the whole life. Efforts were made to keep unintended exposure as low as possible and the cadmium content was measured in whole feed and all individual feed components. The total intake of cadmium during the lifetime of the rats was calculated and the percentage of the cadmium which could be measured in the kidney compared to the calculated total intake was as follows: Control 2.0%, Crushed linseed 0.9%, whole linseed, 1.5%, cocoa 0.7% and cadmium chloride 4.6%. Based on this study it could not be concluded that the bioavailability in rats form whole linseed is lower that for crushed linseed. It was concluded that the bioavailability of cadmium from cocoa was similar or maybe a little lower than the bioavailability of cadmium from linseed.

Keywords: *cadmium, bioavailability, rat, ICP–MS, cocoa, linseed*

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I9
Cancelled

I10
WATER SOLUBLE ELEMENT ANALYSIS OF OLIVE OIL USING TXRF

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The determination of the inorganic profile of edible oils is important for the metabolic role of same elements and to verify possible contamination or adulteration. Element analysis of oil normally requires some time consuming steps like acidic mineralization/destruction of organic matter followed by AAS or ICP determination. Aim of this work was detect some water soluble elements in olive oil using a simple and time-saving Total X Ray Reflection method: metals were extracted from the oil with water, Yttrium or Gallium was added as internal standard and a TXRF spectrometer was used for the determination of the elements that are present in the aqueous phase. About 100 olive oils (extra virgin olive oil, virgin olive oil, refined oil), from the market and from local PDO producers, were analyzed. Mg, P, S, K, Ca, Ba, Cl, Br, Mn, Fe, Ni, Cr, Cu, Al, Zn, As, Ti and Pb were determined and their presence in olive oil, along with the analytical possibilities of TXRF technique are discussed. The dataset were computed using multicomponent statistical analysis trying to enhance possible differences for genuineness or geographical origin assessment purpose

Keywords: *olive oil, TXRF*

I11
SPECIATION OF METALS (ZN, FE, CA AND MG) IN WINE DETERMINED WITH DMT AND AGNES

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The role of metals is crucial in wine, since they affect the nutritional properties and the organoleptic characteristics. The effect of metals, rather than on the total concentration, depends on their speciation or distribution amongst different chemical forms. Few techniques determine the free metal concentration, like the Ion Selective Electrodes (ISE), limited to high concentrations and not commercially available for certain elements like Zn, Mg and Fe. The Donnan Membrane Technique (DMT) [1,2] uses a cation exchange membrane to measure free ion concentration based on the Donnan membrane equilibrium, whereas Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) is a recently developed electroanalytical technique [3,4]. In the literature, AGNES has determined free metals in seawater, freshwater, humic acid solutions, nanoparticles dispersions and wine. DMT has been mainly used in soils and natural waters, but an application to milk matrix has also been reported [5].

In this work, the Donnan Membrane Technique (DMT) has been applied for the first time to determine free ion concentrations (Zn, Fe, Ca and Mg) in a synthetic alcoholic medium and in a commercial red wine (from Raimat, Catalonia, Spain). For the commercial wine, the best configuration includes the concentration of the major ions in the sample (K, Na, Mg and Ca) in the acceptor solution and uses either K or Na as reference ion. The agreement of both techniques, AGNES and DMT, on the determined free Zn concentrations in the same synthetic wine and in the commercial red Raimat wine represents a further cross-validation of these techniques. AGNES has the advantage to the short experimental time while DMT, with much longer equilibration times, detects several cations in the same experiment.

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Keywords: *Zn, Fe, AGNES, DMT, wine*

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I12
DETERMINATION OF SELENIUM IN SELECTED FOOD SAMPLES FROM ARGENTINA BY FI-HGAAS. ESTIMATION OF THEIR CONTRIBUTION TO THE SELENIUM DIETARY INTAKE

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Selenium is an essential micronutrient for humans and it recommended daily intake is set at 55 micrograms per day. However, current information about the content of selenium in soil and food in Argentina and other South American countries is scarce. The main objective of this study was to develop, optimize and validate an analytical method for the determination of total selenium in selected high consumption food: rice, wheat flour, beef, milk and egg. The analytical technique used was an on-line flow injection hydride generation atomic absorption spectrometry (FI–HGAAS) system. Digestion of samples was conducted in a middle of concentrated nitric acid at 90°C until disappearance of nitrous fumes and mixture solution of nitric acid and perchloric acid (4:1). The pre-reduction stage of Se (VI) to Se (IV), necessary for the subsequent reaction of hydride generation, was carried out with hydrochloric acid 8 M. Residues of oxidizing acids and organic matter rema ining from wet digestion of the samples could interfere with the hydride generation reaction. A careful study on the matrix effects becomes critical in order to evaluate the analytical performance. The results obtained showed that the standard addition line slopes (wheat flour, 0.01770 L μg^{-1}; rice, 0.01713 L μg^{-1}; milk, 0.01699 L μg^{-1}; beef, 0.01694 L μg^{-1}; egg, 0.01580 L μg^{-1}) were statistically comparable with the aqueous standard line (0.01733 L μg^{-1}). The variables involved in the generation of selenium hydride were optimized using univariate methods. The method validation parameters were evaluated for each matrix by testing fortification. The robustness of the proposed methodology was studied using experimental design (Plackett-Burman). Average recovery was 96% and the precision was less 5% (n=3) expressed as RSD%. Limits of detection and quantification were 2 and 7 $\mu g\ kg^{-1}$ for milk and 7 and 22 $\mu g\ kg^{-1}$ for other matrices, respectively. The analysis of NIST ref erence material DOLT-3 allowed evaluating the traceability of the methodology. The data obtained allowed to calculate estimates of daily intake of selenium for adults in Argentina. Meat and eggs showed the highest values (in $\mu g\ kg^{-1}$, beef: 42–153; chicken: 62–205; fish: 94–314; canned tuna: 272–282; eggs: 134–217), minor values were found for wheat flour (22–42), rice: (< 22), pasta (47–64) and milk (< 7–9). An estimated intake of 32 $\mu g\ day^{-1}$ and 24 $\mu g\ day^{-1}$ for adult men and women, respectively, suggested a deficient selenium intake, leading to further comprehensive surveys of selenium occurrence in Argentina.

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Keywords: *selenium, food, FI HG-AAS, Argentinean dietary intake*

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I13
TOTAL ARSENIC IN SELECTED FOOD SAMPLES FROM ARGENTINA: ESTIMATION OF THEIR CONTRIBUTION TO INORGANIC ARSENIC DIETARY INTAKE

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Arsenic is a metalloid that is widely distributed in the environment in water, rocks, soil and air, contaminating plants, animals and, consequently, food consumed by humans. Drinking water and food are the primary routes of human exposure. Arsenic toxicity varies according to the chemical form of the element. Inorganic arsenic (i-As) compounds are more toxic than organic compounds. Both forms of inorganic arsenic, i-As(III) and i-As(V), are potentially harmful to health, but the trivalent forms are considered more toxic than the pentavalent forms. The International Agency for Research on Cancer (IARC) classifies inorganic arsenic compounds in Group 1 (carcinogenic to humans) based on sufficient evidence of carcinogenicity in humans. An optimized flow-injection hydride generation atomic absorption spectroscopy (FI-HGAAS) method was used to determine total arsenic in selected food samples (beef, chicken, fish, milk, cheese, egg, rice, rice-based products, wheat flour, corn flour, oats, breakfast cereals, legumes and potatoes) and to estimate their contributions to inorganic arsenic dietary intake. A dry ashing procedure was employed to mineralize the food samples. The limit of detection (LOD) and limit of quantification (LOQ) values obtained were 6 μg kg^{-1} and 18 μg kg^{-1}, respectively, the average recovery for all matrices was 99%, and the precision, expressed as RSD, was 6%. Accuracy was evaluated using dogfish liver certified reference material (DOLT-3 NRC) for trace metals. In this study 117 samples of food commonly consumed in the Santa Fe province, Argentina, were analised. The highest total arsenic concentrations (in μg kg^{-1}) were found in fish (152–439), rice (87–316) and rice-based products (52–201). The contribution of food to the i-As daily intake was estimated by applying conversion factors to total arsenic data from selected foods (14% for fish and 70% for other foods) and the mean consumption per day. The major contribution of i-As to Argentinean dietary intake was attributed to wheat flour, including its proportion in wheat flour-based products (breads, pasta and cookies), followed by rice; both foods account for close to 50% and 25% of the intake, respectively. The contribution from beef was due to its high consumption, although it did not have quantifiable arsenic levels. Other foods showed significant i-As concentrations, such as fish, breakfast cereals and rice-based products, although they had a minor or no significant contributions due to the low amounts consumed. Finally, i-As intake from selected foods was estimated as 10.7 μg day^{-1}, which is significantly lower than that obtained from drinking water in vast regions of Argentina that have natural arsenic groundwater contamination.

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Keywords: *inorganic arsenic, food, FI HG-AAS, Argentinean dietary intake*

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I14
APPLICATION OF FACTORIAL DESIGN TO STUDY THE INFLUENCE OF SAMPLE TREATMENT PARAMETERS IN THE DEVELOPMENT OF A NEW METHOD FOR TRACE ELEMENTS DETERMINATION IN RAW MILK

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Background: Milk is a widely consumed food, particularly by infants. For this reason, its safety and quality standards are a matter of great concern and close monitoring. International organizations have established very low limits for contaminants that can be present in trace levels in milk. Therefore, the development of a simple, rapid and sensible method for quantification of trace elements in milk is of critical importance. For preliminary studies, factorial design is a very powerful tool. Even with a small number of experiments, it can infer the variables that exert significant influence in the analytical method and the interaction between them.
Objective: The aim of this study was to apply experimental design to assess the influence of the main parameters involved in the development of an alternative method for the determination of the trace elements arsenic (As), cadmium (Cd), lead (Pb) and selenium (Se) in raw milk by inductively coupled plasma mass spectrometry (ICP-MS), using ultrasound-assisted acid extraction. Method: One-milliliter aliquots of raw milk were spiked with As, Cd, Se (50 μg L/L) and Pb (20 μg/L). Ultrasound-assisted acid extractions were performed using formic acid and an ultrasonic probe (Unique). In order to evaluate the effect of the factors “acid concentration” (AC) and “sonication time” (ST) on the elements recovery, a two-level factorial design was employed. The levels selected for the variables were: 12.5 or 50% and 30 or 90 seconds for AC and ST respectively. Trace elements concentrations were determined by ICP–MS (Agilent’s 7700x).
Results: The effect of the variable AC was significant for all the inorganic contaminants. For the elements As, Cd and Pb, within the studied limits, lower acid concentrations resulted in higher recovery rates. For the element Cd, the opposite effect was observed: lower acid concentrations resulted in lower recovery rates. However, recovery percentages closer to 100% were observed for the low level of AC. The effect of the variable TS was significant only to the elements As and Pb, indicating that an increase in the sonication time leads to higher recovery rates. Nevertheless, the magnitudes of the effects found for this variable were considerable smaller than the magnitudes found for AC. Moreover, even in short sonication times, the recovery percentages were satisfactory.
Conclusion: The parameter “acid concentration” had the greatest influence on the elements recovery rate. Since the desired outcome was to obtain recovery percentages closer to 100%, lower acid concentrations showed the best results for all the elements tested. In a future study, a central composite design (22 + center points + axial points) will be used to optimize the sample treatment parameters for simultaneous determination of the four inorganic contaminants.

Keywords: *trace elements, experimental design, raw milk, radip method, ICP-MS*

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I15
BOTANICAL DISCRIMINATION OF SICILIAN RED WINES BY MULTI-ELEMENTS ANALYSIS

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33 red wines from *Vitis vinifera* var. Nero d'Avola (19 samples) and Syrah (14 samples) grown in Sicily during 2014 have been analyzed.The levels of 16 mineral elements (K, Ca, Mg, Na, Zn, Fe, Mn, Cu, Cr, Pb, Ni, Co, Se, As, Cd and Sb) were determined by Inductively Coupled Plasma Optical Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry. Validation studies were carried out and all samples were analyzed in triplicate, in batches with blank samples and standards, to assure high results quality standards. In addition, also some physical and chemical parameters were determined according to Commission Regulation (EEC) No. 2676/90. The aim was to assess which parameters could allow discriminating the different botanical origin of the analyzed samples. For this purpose statistical methods such as Mann-Whitney U test, Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA) were applied to obtained data. Normally K, Mg, Ca and Na were the most abundant minerals ranging between 748.46–1975.68, 117.58–198.57, 68.41–150.45 and 10.30–170.55 mg/L, respectively. Zn, Fe, Mn and Cu contents ranged from 0.12 to 8.63 mg/L; Pb and Ni varied between 10.00 and 169.11 µg/L, while Cr was from 10.45 to 82.00 µg/L. Co, Se and As contents were from 1.01 a 11.59 µg/L. Cd and Sb were detected only in some samples with maximum value of 0.78 and 0.92 µg/L, respectively.The levels of the mineral elements are, generally, in compliance with the Maximum Residue Level fixed by current standard, but some exceptions were observed for Zn and Cu. Moreover, it was verified that the values of the physical and chemical parameters are in accordance with the limits set by current legislation, but does not affect relevantly the discrimination between samples obtained from var. Nero d'Avola to those obtained from var. Syrah, except for the value of the total acidity that was significantly higher in wines from var. Nero d'Avola. Significant differences (at $p < 0.05$) were observed in K, Mg, Zn, Cu, Cr, Ni, As, Cd and Sb mean levels among wines of different botanical origin, with samples from var. Nero d'Avola having the highest Zn, Cr, Ni, As and Cd content, while samples from var. Syrah the highest of the remaining.Using the PCA the discrimination between wines obtained from var. Nero d'Avola to those obtained from var. Syrah was achieved, and also the results of CDA indicate that the 100% of total samples are correctly classified. It is proved that it is possible related red wines to varieties of *Vitis vinifera* according to their mineral contents. This research contributes to the studies to determine the wine botanical origin.

[1] Regulation (EEC) No 2676/90 determining Community methods for the analysis of wines.
[2] D.M. 29 dicembre 1986. Caratteristiche e limiti di alcune sostanze contenute nei vini.
[3] Di Bella G., Lo Turco V., Potortì A.G., Bua G.D., Fede M.R., Dugo G.mo. 2015. Journal of Food Composition and Analysis 44:25–35.

Keywords: *wine; mineral elements; food analysis; multivariate statistics; botanical origin*

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MYCOTOXINS, MARINE AND PLANT TOXINS

(J1 – J58)

J1
SURVEY OF ALTERNARIA TOXIN CONTAMINATION OF GERMAN FOOD USING A RAPID HPLC–MS/MS APPROACH

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Fungi of the genus Alternaria occur worldwide and infect various agricultural crops including grain, fruit (apple), solanaceous herbs (tomato, potato) and sunflowers. Besides their phytotoxic – and thereby detrimental - activity, they produce a variety of about 70 mycotoxins such as tenuazonic acid (TeA), alternariol (AOH), alternariol mono methyl ether (AME), altenuene (ALT) and isoaltenuene (isoALT), altertoxins (ATX), tentoxin (TEN) and AAL toxins (AAL). These toxins show a broad range of adverse effects, including teratogenic, cytotoxic and mutagenic effects. Up to date, no legal maximum levels or guidance levels for alternaria toxins in food or feed have been set by the European Union. This is - besides the incomplete toxicological data - due to the scarce occurrence data. We developed a simple and fast LC-MS/MS method for the quantification of TeA, AOH, AME, ALT, isoALT, ATX I, TEN, AAL TA1 and TA2, covering the mainly occurring toxins in foodstuff. The method relies on a single-step extraction and subsequent dilution of the raw extract without any further laborious purification and was validated for five different food matrices. The analysis of about 160 food samples from German retail revealed the widespread contamination of fruit juices, tomato products, vegetable oils, sunflower seeds and bakery products with TeA, AOH, AME and TEN. Almost all samples were positive for one or more toxins. TeA occurred in levels of 20–2300 µg/kg, AOH in levels from 0.8–25 µg/kg, AME in levels of 0.1–170 µg/kg and TEN in levels between 0.5 and 750 µg/kg. This study adds valuable occurrence data and thereby helps to carry out an accurate risk assessment.

Keywords: *alternaria, mycotoxin, multi method, mass spectrometry, isolation*

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J2
DEVELOPMENT OF A SENSITIVE, EFFICIENT AND RAPID MYCOTOXIN MULTIRESIDUE METHOD IN FOOD BY SPE AND DSPE METHODOLOGY AS ALTERNATIVE TO EXPENSIVE IAC TECHNOLOGY

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Every year approximately 20% to 25% of crops worldwide are contaminated by fungi. Due to global warming and climate changes the contamination of crops by fungi is expected to increase in the coming years. Mycotoxins have an acute and also chronical toxic impact on human beings and livestock. The most occurring species are of Penicillium, Aspergillus and Fusarium genus, which produce mycotoxins in certain growth stages under specific climate conditions. In consideration of health hazards and the legal requirements there is a demand for sensitive analytical procedures for the determination of mycotoxins in food and feed. The aim of our study was the development of a sensitive multiresidue method (MRM) for the analysis of a wide scope of fusarium toxins, aflatoxins, ochratoxin A, alternaria toxins and ergot alkaloids in food is presented. The strategy regarding the optimization of selectivity and sensitivity of DSPE and SPE steps are described. Following a QuEChERS-like extraction step using buffered conditions the extract is subjected to two subsequent cleanup modules. In the first module co-extractives are removed in DSPE mode using a specific subset of normal phase and reverse phase sorbents. In the second cleanup step the toxins of interest are further purified in retentive SPE mode. A change in the solvent composition of the mobile phase during the subsequent cleanup steps was crucial for the efficacy in the removal of co-extractives while retaining the mycotoxins of interest. The analytical technique was validated according to DIN 32645. The LOQs determined for example for aflatoxins were in the range of 0.01 ppb and comparable to limits achievable by actual IAC methods. This selective and robust MRM protocol represents a valid alternative to the cleanup with affinity chromatography. The multiresidue method is applicable to a wide scope of commodities like cereal products, dried fruits, oilseeds and nuts and infant food.

Keywords: *mycotoxins, fusarium toxins, alternaria toxins, DSPE, SPE*

J3
MULTI-ANALYTE MYCOTOXIN AND ERGOT ALKALOID ANALYSIS USING µLC–MS/MS

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Mycotoxins and ergot alkaloids are secondary fungal metabolites. Mycotoxins can be produced by filamentous fungi such as species of the genus Fussarium and Aspergillus. Ergot alkaloids are produced by certain species of the genus Claviceps. The major groups of mycotoxins are trichothecenes type A (i.e. T2 and HT2) and type B (nivalenol and deoxynivalenol), aflatoxins, fumonisins and ochratoxins. And then there are also new emerging mycotoxins such as the fusarium toxins beauvaricin and enniatins. The presence of mycotoxins and ergot alkaloids in various crops such as maize and grains like rye, barley and wheat is influenced by climate and storage conditions. These possible toxin containing commodities may and up food and feedstuffs like for example cereals, pasta or animal feed. When consumed these toxins may have severe direct or long-term effects such as liver and kidney failure or carcinogenic and/or genotoxic effects. Therefore, to ensure food and feed s afety monitoring programs for these toxins in various commodities have been established. The presence of these toxins in for example animal feed in most of the more advanced laboratories is done by multi-analyte LC–MS/MS methods. Typically UHPLC in combination with the latest generation tandem mass spectrometers which are capable of detecting a single transition within 5 ms. The latter in combination with UHPLC enables the possibility to analyse up to a few 100 compounds in a single analysis. In order to extract these numbers of compounds or in this case toxins with different physicochemical properties a generic extraction procedure are used. In this case a generic QuEChERS with acetonitrile and acetic acid as extraction solvent was used. Animal feed compositions differ therefore matrix ionisation effects, ion suppression and enhancement, greatly differ from sample to sample. To have a correct quantitation of the amount of toxins in a sample standard addition is needed. This is a laborious task. Furthermore, by introducing a relative dirty matrix in the MS may als o cause contamination of the system and this requires more maintenance. One of the solutions to reduce matrix effects and reduce the contamination of the MS is moving towards microfluidic chromatography (µLC). With µLC smaller quantities of the sample are introduced on the column, typically 1–2 µL in a flow rate of 20–40 µl/min. This reduces the contamination en enhanced the sensitivity. For the determination of over 40 different toxins in animal feed an µLC–MS/MS method was developed. The method was developed using the Eskigent µLC in combination with the ABSciex 6500 Qtrap mass spectrometer. During the method development optimal injection volume, flow rate and column temperature was established. The developed µLC method was compared with the conventional LC–MS/MS method to observe the pros and cons of the µLC application. Results of this study will be presented.

Keywords: *microLC, ergot alkaloids, mycotoxins*

J4
STRUCTURAL CHARACTERIZATION AND PROPOSED MECHANISMS FOR THE AFLATOXINS DEGRADATION BY A SELECTED MICROBIAL LACCASE

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The production of the major aflatoxin isorforms by selected fungal strains, including Aspergillus flavus and Aspergillus parasiticus as well as their degradation by a laccase from Coriolus hirstutus were investigated. A. parasiticus was found to be the most appropriate one in term of its capacity to produce the highest aflatoxins level. A reverse-phase/high-performance liquid chromatography (RP–HPLC), at 365 nm, was used to separate and characterize the aflatoxins, where AFG2, AFG1, AFB2 and AFB1, were eluted at 3.70, 4.20, 4.80 and 5.08 min, respectively. The aflatoxins degradation by microbial laccase (PPL), from Coriolus hirsutus, was investigated. Using the major aflatoxin isoforms, AFB1, AFB2, AFG1 and AFG2, as substrates for the PPL, the residual aflatoxins as well as the degradation end products was analyzed by RP–HPLC. The results showed an enzymatic degradation rate of 38.2, 30.1, 76.4 and 100% for AFB1, AFB2, AFG1 and AFG2, respectively. In addition, the structural characterization of the purified aflatoxins, from Aspergillus parasiticus, and their enzymatic degradation end products were investigated, using of Fourier-transform infrared spectroscopy (FTIR) and mass spectrometry (MS). The results showed that the degradation of the aflatoxins resulted in the formation of a wide range of several end products, where the most abundant one was the molecular ion peak at *m/z* 327.254, 205.069, 205.069 and 196.656 for AFB1, AFB2, AFG1 and AFG2, respectively. The FTIR and MS analyses suggested that the mechanisms of aflatoxins enzymatic degradation could be the epoxydation, hydroxylation, O-demethylation, dehydrogenation, dehydratation, reduction of the double bond and keto-reduction a well as the loss of ketone, oxygen, carbon and methyl molecules which could lead to the modification of either furfuran moiety, coumarin or the lactone ring as well as to the formation of nontoxic products.

Keywords: *aflatoxins, degradation, laccase, structural analyses, mechanism of degradation*

J5
AN ENVIRONMENTALLY FRIENDLY MULTI-EXTRACTION METHOD FOR SCREENING OF MYCOTOXINS

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Mycotoxins are toxic secondary metabolites produced by a broad range of fungal species. As these toxins are frequently found in agricultural products, many nations have set maximum limits for the most relevant substances. Due to these regulations, rapid screening methods are required to enable industry and authorities to test for mycotoxins. The fast and simple extraction of mycotoxins from agricultural commodities is a crucial step in the development of rapid test systems. Due to the low solubility of most mycotoxins in aqueous solutions the extraction is usually performed with organic solvents like methanol or acetonitrile. Especially for on-site screening methods, such as fast and simple Lateral Flow Devices (LFDs), untrained people are exposed to harmful substances when using organic solvents. Moreover, these compounds can be considered as environmentally malign when larger quantities are used for sample extraction. The reduction or the complete replacement of these substances is of great interest and importance for the future. Therefore, a unique water-based method for the simultaneous extraction of numerous mycotoxins has been developed: WATEX…WATer EXtraction The WATEX method is using an extraction buffer, provided in water-soluble bags, so called “Buffer Bags”, for fast and easy handling. One of such a Buffer Bag is added to a milled grain sample and just water is necessary for the extraction. Therefore, the use of large amounts of toxic organic solvents is obsolete and the disposal of extracted samples is much easier. This extraction provides a substantially higher safety for human and environment as well as a reduction of costs. Once a sample has been extracted using this solvent free approach, the extract can be used for the rapid screening of the following mycotoxins: Aflatoxins, Deoxynivalenol, Fumonisins, Ochratoxin A and Zearalenone. Validation studies according to the United States Department of Agriculture (USDA) GIPSA (Grain Inspection, Packers & Stockyards Administration) criteria could successfully be performed using the developed WATEX method; the determined limits of quantification were either equal or below the required European or US limits. Within this presentation highly promising results for the five above mentioned mycotoxins will be shown. Sensitive rapid tests for controlling maximum allowed limits were developed as easy-to use on-site screening methods.

Keywords: *lateral flow devices, rapid tests, waterbased extraction, mycotoxins*

J6
LC-MS/MS MULTI-MYCOTOXIN ANALYSIS – A NEW APPROACH IN ROUTINE ANALYSIS

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The popularity of Liquid Chromatography – Tandem Mass Spectrometry (LC–MS/MS) technology in mycotoxin (MT) analysis is constantly increasing. Laboratories use these methods in routine testing, where it is important to meet ISO 17025 standards. Unfortunately, interferences from matrix components influence the analyte ionization and lead to incorrect LC–MS/MS results. A major drawback is observed variations of ionization efficiencies between sample matrices and the pure standard calibrants used for quantification. Hence, the mass spectrum shows different signal intensities and the comparison of the analyte peak with a standard calibration curve for the calculation of concentrations leads to wrong results. Internal standards (IS) can be used to overcome these impairing effects.
Isotope labeled standards, like e.g. ^{13}C-labeled MTs, with one or a number of constituting carbon atoms replaced by the stable isotope ^{13}C, still have the same characteristics as their ^{12}C analogues. They elute at the same retention time in chromatography but show a different mass what easily distinguishes them from their ^{12}C analogues. Detected by mass spectrometry, the ^{13}C peak represents the known amount of ^{13}C labeled MT added to the sample. Subsequently, this peak is used to accurately calculate the unknown amount of the ^{12}C MT.
To meet ISO 17025 standards and fulfill EU criteria highly sensitive MT detection methods are required. To enable the detection of MTs at very low detection limits the implementation of a sample cleanup step in the LC-MS/MS method significantly improves the quality of the obtained results. Romer Labs® has developed a novel rapid multi-MT clean-up to increase sensitivity by reducing the matrix effects. The combination of 13C-isotope-labelled IS and multitoxin clean-up leads to a novel and innovative analysis procedure applicable to analyze a wide variety of matrices.
This talk presents a newly developed LC–MS/MS multitoxin testing concept covering all EU regulated MTs including Aflatoxins, Fumonisins, DAS, T-2 and HT-2 toxin, Nivalenol, Deoxynivalenol, 3- and 15-Acetyldeoxynivalenol, Ochratoxin A, Fusarenon-X and Zearalenone for the application in routine testing labs. Various food and feed samples, including complex matrices were analyzed with this method combining MT cleanup and 13C-labeled IS. LODs ranged from 0.07 µg/kg to 0.66 µg/kg for the Aflatoxins and Ochratoxin. For the various Type A and B Trichothecenes and for Zearalenone LODs ranged from 1.05 µg/kg to 13.10 µg/kg. The % RSD of multiple repetitions of spiked samples was less than 15% overall, with most data showing % RSD less than 10%. Recoveries of the MTs from spiked matrices varied by MTs; however all recoveries were above 70%.
These results clearly reveal the importance of using ^{13}C-labeled IS and multitoxin cleanup in routine LC–MS/MS analysis to optimize the results required by EU legislation and to obtain a workflow for easy implementation in routine laboratories.

Keywords: *LC–MS/MS, routine analysis, multimethod, mycotoxins*

J7
DEVELOPMENT OF SENSITIVE EXPRESS IMMUNOASSAYS FOR MYCOTOXINS

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Mycotoxins are highly important controlled toxic contaminants of food stuffs. Despite the diversity of existing methods for their detection, there is a lack of approaches that combine sensitivity, rapidity and simplicity of testing (including sample preparation). We have developed new formats of lateral flow immunoassay (LFIA) and enzyme-linked immunosorbent assay (ELISA) for mycotoxins (aflatoxin B1, ochratoxin A, T-2 toxin, and zearalenone) allowing to reach such combinations. The proposed format of LFIA is based on the application of non-labeled specific antibodies and anti-species antibodies conjugated with gold nanoparticles. This approach provides efficient competitive binding of target mycotoxin in the sample and by this way revealing lower concentrations of the analyte. The comparison with traditional LFIA for aflatoxin B1 demonstrated 10-fold improvement of the assay sensitivity; the reached limit of detection (LOD) for modified LFIA is equal to 20 pg/mL. The application of the same approach for T-2 toxin and zearalenone also demonstrated its efficiency; LODs are equal to 3 and 2 ng/mL, correspondingly. The application of magnetic immunosorbents is proposed to improve the ELISA parameters. These carriers allow to carry out immune interaction in a large volume of sample without diffusion limitations and then to separate the formed complexes rapidly and characterize the analyte binding. In contrast to common immunopurification, the proposed assay is implemented without elution stages. It was shown that pseudo-homogeneous immune reaction reached equilibrium in 5 min and by this way the total ELISA duration was reduced to 25 min (for the case of aflatoxin B1 detection). Due to concentration of the analyte from a large initial volume to final microplate well the LOD of the aflatoxin B1 ELISA decreased to 2 pg/mL. An additional advantage of magnetic immunosorbents is high stability of immobilized antibodies to organic solvents (up to 30% of methanol) that are used for mycotoxins extraction. Thus, extracts of agricultural products may be used for the assay with minimal dilution, th ereby reducing the loss of the assay sensitivity. The developed techniques were validated in testing corn and barley samples that were characterized by chromatographic methods. The compared assays demonstrated high correlation of results, the degree of mycotoxins revealing by the proposed immunotechniques was not less than 90%. The results obtained indicate the prospects of the proposed immunoassay formats for mass screening control of agricultural products, food and feed for mycotoxin contamination.

Keywords: *mycotoxin, lateral flow immunoassay, magnetic concentration*

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J8
MULTIRESIDUE COMPATIBLE APPROACH FOR AFLATOXIN M1 DETERMINATION IN RAW MILK BY UHPLC-MS/MS

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Mycotoxins are along with pesticides the major contaminants in crop-products used as animal feedstuff in farms. The major concern in milk is the presence of aflatoxin M1 (AFM1) which is the hydroxylated metabolite of aflatoxin B1, a toxin produced by *Aspergillus* genus moulds that may grow in seeds and grains during production or storage of crops. The high toxicity of AFM1 due to its carcinogenic potential led to the establishment of very low MRLs (0.5 and 0.05 μg L^{-1} set by US FDA and EU respectively). These low levels have restricted appropriate analytical methods for milk monitoring and enforcement purposes to be very sensitive and selective, presenting analytical complexity due to matrix interferences and difficulties to be included in multiresidue approaches.
The well-known QuEChERS approach for pesticide extraction and purification from fruits, vegetables and many other foodstuffs could be used for the extraction of AFM1 from milk. In this case two aims may be afforded: to replace the official single method based on immunoaffinity columns (IAC) and to make it compatible with a large scope pesticide multiresidue extraction.
The aim of this work was to optimize a simplified and reliable extraction procedure based on QuEChERS for the multiresidue analysis of AFM1 and pesticides in raw milk with a sensitive and rapid determination by UHPLC-MS/MS.
Since the use of QuEChERS has not been much published for the extraction of AFM1 from milk, a procedure for pesticide extraction from this complex matrix was adopted for our purpose. The study and optimization of critical variables was performed, including the acetonitrile-partitioning agitation time and the amount of C18 in the clean-up step. Method performance for determining 63 pesticide compounds and AFM1 was evaluated through recovery studies and calculating validation figures of merit under SANCO/12571/2013 guidelines.
The presentation will be focused on the performance description of the AFM1 determination in the multi-compound methodology context. Good recovery results were obtained at the EU regulated level of 0.05 μg L^{-1}(average 86%) for AFM1. Acceptable intra and inter-day repeatability was achieved (RSD <15%). Limits of determination obtained for AFM1 demonstrate compliance with established MRLs both in Argentina and at international levels. The method was applied for the analysis of 80 real samples from the agricultural central region of Santa Fe in Argentina.

Keywords: *aflatoxin M1, QuEChERS, multiresidue, UHPLC–MS/MS*

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J9
AFLATOXIN CONTAMINATION ALONG THE PEANUT PASTE PRODUCTION CHAIN IN CÔTE D'IVOIRE

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A previous study carried out within the framework of the EuropeAid project 3CIvoire (2011–2014) for determining multi-mycotoxin occurrence in rice, corn and peanut products collected in the main markets of Abidjan, Bouaké and Korhogo in Côte d'Ivoire showed that peanut paste represented the highest risk to consumer health. This work aimed to determine aflatoxin contamination throughout the peanut paste production chain in Côte d'Ivoire. A survey of peanut producers and traders in the region of Korhogo was conducted to build and check the flowchart, detailing practices from the production of peanuts in the field to marketing of peanut paste. Based on the flowchart, 65 samples were collected in nine points: 35 peanut pod samples (10 harvested in the field, 10 collected after drying in the field, 10 collected after drying and 5 after storage in village), 15 shelled peanut samples (5 stored by wholesalers and 5 stored by retailers, 5 roasted) and 15 past e samples (5 freshly obtained after grinding and 10 collected in markets). The samples were analysed for the determination of aflatoxins by HPLC–FLD and water activity. Potentially aflatoxigenic Aspergillus spp. were also enumerated and isolated on AFPA medium. Forty two samples (i.e. 65 %) were contaminated by aflatoxins with 55% and 52% exceeding the European Union (EU) limits of 2 µg/kg for aflatoxin B1 (AFB1) and 4 µg/kg for total aflatoxins (AFB1+AFB2+AFG1+AFG2), respectively. The levels of AFB1 and AFT varied from 0.4 to 477.8 µg /kg and 0.4 to 541.1 µg /kg, respectively. Peanut pod samples collected in the field after harvest and after drying were not contaminated by aflatoxins. At the other collection points, 70% to 100% of the samples were contaminated by aflatoxins with 75% to 100% of these samples having levels of AFB1 and AFT exceeding the EU limits. The water activity was higher than 0.85 in the samples collected in the field and less than 0.72 in the sample s collected at the other steps of the peanut paste production chain. Potentially aflatoxigenic Aspergillus were enumerated between 2 and 5.2 log (10) cfu/g, but none was found in the samples collected at harvest in the field, after roasting and after grinding. Two hundred and fifty seven potentially aflatoxigenic Aspergillus strains have been isolated. A study is currently underway for species identification and characterization for aflatoxin production.

Keywords: *peanut, aflatoxins, aspergillus, Côte d'Ivoire*

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J10
IMPROVED QUANTITATIVE ANALYSIS OF PESTICIDES AND PLANT TOXINS IN HERBAL TEAS THROUGH REDUCTION OF MATRIX SUPPRESSION AND INTERFERENCES BY MULTI-DIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED TO ESI-MS

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Herbal teas and food supplements represent a challenging matrix for the quantitation of residues and contaminants with LC/MS based methods. Ion suppression of the analytes by co-eluting matrix compounds or isobaric interferences hamper the accurate quantitation and increase the number of false positives and false negatives. There is growing concern about the occurrence of hepatotoxic pyrrolizidine alkaloids (PA) in these products. PAs are secondary metabolites of plants and to date there are more than 800 compounds known of which many are isomeric and due to the common structure elements behave similar in chromatography and even in MS/MS fragmentation. Two-dimensional liquid chromatography (2D–LC) has been demonstrated to greatly enhance separation performance compared to conventional 1D–LC. The benefits for accurate quantitation of pesticides and PAs in complex matrices gained by coupling a second dimension separation directly to the standard LC–MS method i s investigated. Extraction of the plant matrices was done following the QuEChERS protocol or with acidified water and methanol. Crude extracts were either injected directly or were cleaned up with SPE. Refined extracts were evaporated to dryness and reconstituted with mobile phase. PAs and pesticides were analyzed using a conventional reversed phase UHPLC experiment and were analyzed using 2D–LC, both coupled to ESI–MS. In addition to full comprehensive 2D–LC, multiple heart-cutting (MHC) was used which focusses on interesting regions of the 1D chromatogram and is predominantly used for targeted, quantitative analysis. The peak parking functionality of MHC breaks the link between 1D and 2D time scales, which allows for using longer 2D–cylces and columns with higher separation efficiency. Additionally it allows for flow rates which are more acceptable for mass spectrometry detectors. The approach of both parking individual analytes into single parking loops as well as using multiple parking positions to scan specific regions of the chromatogram are demonstrated. Using the MHC approach with 12 sampling loops, and a 2nd second reversed phase dimension with slightly modified conditions (Acetonitrile instead of Methanol, changed gradient slope) produced different selectivity between 1D and 2D resulting in a separation of the analyte from the matrix. In rooibos extracts were several PAs (e.g. retrorsine, senecionine and their N-oxides) were interfered by isobaric substances in 1D, MHC 2D–LC achieved full separation of the components. The overall analysis time was marginally longer but the suppression effects were significantly reduced. For several compounds, for which strong suppression was observed, quantitation limits were improved by a factor of 10. A further result was the change in adduct formation in 2D compared to 1D. While several compounds in the 1D analysis predominantly formed sodium adducts, the major adduct after 2D separation was the [M+H] species.

Keywords: *multidimensional separation, multiple heart-cutting, reduced ion-suppression, pyrrolizidine alkaloids, pesticides*

J11
ERGOT ALKALOIDS: FROM WITCHCRAFT TILL IN SILICO MODELS. MULTI-RECEPTOR ANALYSIS OF ERGOTAMINE METABOLITES

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The term Ergot is referred to the protective kernel (i.e. sclerotium) produced during resting stage of ascomycetes belonging to Claviceps genus that replaces seeds of susceptible cereals and plants intended for human and animal diet. It contains various composition of tryptophan-derived toxins defined ergot alkaloids (EAs). Since sclerotia can be harvested and milled together with cereals, EAs represent a source of food and feed contamination after breakage and spreading of mycotoxins into the various milling fractions. The effects of EAs have been known since the Middle Ages and, currently, it has been recognized the occurrence of gangrenous and convulsive ergotism. Nowadays, it is known that EAs action is manly mediated by the interaction with alpha-adrenergic, dopaminergic and serotonergic receptor classes. In spite of the wealth of studies on synthetic compounds and drugs, further data are needed on metabolism and receptors-binding for common EAs in food, as recently stated also by the European Food Safety Authority. Unfortunately, the systematic in-depth analysis of interaction between the entire spectrum of metabolites and the array of targets is hardly achievable through the unbiased experimental analysis. The use of in silico techniques may be an effective choice to smartly drive the upstream selection of strong candidates for more detailed workbench investigations. Focusing on ergotamine as the case study, the present work was aimed at assessing whether an in silico approach based on docking simulations and re-scoring procedures can be an effective tool to investigate the interaction between multiple serotonin receptors and a wide set of ergotamine metabolites. In the effort to profile the overall effect of human metabolism on ergotamine action all the metabolites identified so far were collected from the literature (i.e. 10 molecules). Most likely, they do not complete the truly circulating array of metabolites, thus, with the end to assess more widely the effect of chemical modific ation by human metabolism, a series of metabolites has been computed (i.e. 22 molecules). Starting from crystallographic structure of serotonin receptor 2B (5HT-2B), 5HT-2A and 5HT-2C models were derived by using homology modeling. All the three models underwent validating procedure and then the capability of both sets of metabolites to interact with the validated binding sites was computed. It is worthy to note that the most of metabolites were predicted as able to interact with targets (notably, glycosylated compounds were not included), albeit they showed a certain degree of receptor-specificity. Thus, the need for more detailed investigation has been ultimately suggested to verify the possible retaining of activity for the most of phase-I metabolites. Furthermore, in silico modeling showed to be a powerful tool for the hierarchical prioritization of molecules and receptors in the view of supporting the rational design of future workbench experiments.

Keywords: *ergot alkaloids, serotonergic activity, in silico analysis, homology modeling, ergotamine metabolites*

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J12
ANALYSIS OF LIPOPHILIC MARINE BIOTOXINS IN FRESH, PROCESSED AND CANNED BIVALVES BY LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY: A QUECHERS APPROACH

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Analysis of lipophilic marine biotoxins in fresh, processed and canned bivalves by liquid chromatography coupled to high resolution mass spectrometry: a QuEChERS approach. Maximum concentration limits for some marine biotoxins (MBTXs) in bivalves have been set up in many countries to protect public health. In the European Union (EU), Regulations No 853/2004 [1] and EU 786/2013 [2] establish the permitted limits of lipophilic MBTXs in fresh bivalve mollusks. EU legislation does not consider canned and processed products, although, it has been shown that processes such as industrial steaming may produce an important increase of the diarrheic shellfish poisoning toxicity [3]. This weakness in the EU legislation has been partially overcome by including some advice for processed and canned mussels analysis in the latest Standard Operational Procedure from the EU Reference Laboratory for Marine Biotoxins (EU-RL-MB) [4]. Advice has been focused in sample pre-treatment for some processed mussels, making sample pre-treament longer and more tedious and not supplying guidance for the analytical difficulties arisen when routine analysis of these complex samples. In response to the wide variety of samples that should be analyzed (fresh, processed and canned bivalves) and considering that new analytical methodology is available, the laboratory of the Agència de Salut Pública de Barcelona (LASPB) has developed a new method for the analysis of lipophilic MBTXs (okadaic acid, dinophysistoxins, azaspiracids, pectenotoxins, yessotoxins, spirolids). The method is based on the use of QuEChERS and liquid chromatography coupled to high resolution mass spectrometry using and internal standard for matrix-matched calibration [5]. With this methodology the matrix effect is minimized and specificity is enhanced due to the use of high resolution mass spectrometry. The method is currently used in the LASPB for official control programs and it has been included in the scope of the accreditation following ISO/IEC 17025 requirements. Recently, it has been successfully used to study migration and concentration processes in canned and cooked steamed mussels.

[1] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 Laying Down Specific Hygiene Rules for Food of Animal Origin,OJ L 139, 30.4.2004, p. 55.
[2] Commission Regulation (EU) No 786/2013 of 16 August 2013 Amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Coun-cil as Regards the Permitted Limits of Yessotoxins in Live Bivalve Molluscs, OJL 220, 17.8.2013, p. 14.
[3] J. Blanco, et al. Food Chemistry 2015,177, 240-247.
[4] http://aesan.msssi.gob.es/CRLMB/docs/docs/metodos_analiticos_de_desarrollo/EU-Harmonised-SOP-LIPO-LCMSMS Version5.pdf (accessed 14 April, 2015).
[5] A. Rúbies, et al. Journal of Chromatography A 2015, 1386, 62-73.

Keywords: *lipophilic marine biotoxins, HRMS, fresh, processed and canned bivalves, QuEChERS*

J13
BIOCHIP ARRAY TECHNOLOGY FOR THE RELIABLE PERFORMANCE OF MULTI-MYCOTOXIN DETERMINATION IN ANIMAL FEED MATERIALS

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Introduction. Mycotoxins are a group of naturally occurring toxins produced by certain fungi, commonly known as moulds, which are harmful to humans, domestic animals and livestock. Mycotoxins are found in a wide range of foods and feeds, particularly in areas with climates of high temperature and humidity. Factors of both a scientific and socio-economic nature largely influence the establishment of mycotoxin limits and regulations. Currently, chromatographic, spectrometric and immunoassay based techniques are used for the detection of these toxins. Biochip array technology allows the simultaneous screening of multiple analytes from a single sample, which consolidates testing and reduces the quantity of samples to be assessed by confirmatory analysis. This study evaluates a biochip array kit which enables the screening of multiple mycotoxins from a single sample. Animal feed samples from a proficiency testing scheme were analysed.
Methodology. Simultaneous competitive chemiluminescent immunoassays arrayed on the biochip surface and applied to the Evidence Investigator analyser, were employed. The system incorporates dedicated software to process and archive the multiple data generated. Authentic feed sample comparisons (n=8) with LC–MS/MS were performed. Moreover, assigned samples (animal feed, wheat flour, maize) from a proficiency testing scheme (FAPASR) were assessed (n=11).
Results. The biochip array kit showed a broad specificity profile: aflatoxins, ochratoxin A, fumonisins, trichothecenes, zearalenone (including metabolites), ergot alkaloids and paxilline were detected. The detection limits were at or below the regulatory limits in feed. Initial authentic feed sample comparisons (n=8) with LC–MS/MS showed 100% agreements for all analytes. When animal feed, maize and wheat flour samples from the performance assessment testing programme were tested (n=11), the values obtained with the biochip based immunoassays fell within the z-score range ($-2 \leq z \leq 2$); in addition two maize samples presented multiple mycotoxins, which were simultaneously detected with the biochip array.
Conclusions. The results indicate that the biochip array kit enables the detection of multiple mycotoxins from a single sample. The assessment of authentic animal feed samples showed a percentage agreement of 100 with LC–MS/MS. The analysis of assigned samples from FAPASR indicated that the biochip array method is fit for purpose as all the values fell within the established z-score range of the programme. Moreover, with the biochip array contamination with multiple mycotoxins can be detected from one single sample. This biochip array kit represents a useful and reliable multi-analytical tool to increase the screening capacity in test settings.

Keywords: *biochip array, mycotoxins, performance, animal feed, screening*

J14
BIVALVE MOLLUSCS AVAILABLE IN POLAND AS A POTENTIAL SOURCE OF MARINE BIOTOXINS

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Meat of mussels may contain bacteria (such as Salmonella, E. coli or Staphylococcus) or viruses, but also a number of chemical compounds such as heavy metals, pesticides, drug residues and marine biotoxins. Marine biotoxins are a group of natural toxins which may accumulate in seafood. More of these toxins are produced by marine algae (phytoplankton, including diatoms and dinoflagellates) and can accumulate in fish, shellfish or molluscs if they ingest these algae. In molluscs, the concentration of biotoxins in hepatopancreas is even several times higher than in the meat. This is particularly true of lipophilic biotoxins. Consumption of molluscs is growing up in Poland. The safety of shellfish can be compromised by contamination with marine biotoxins. They include paralytic shellfish poisoning (PSP), diarrheic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP). The objective of this study was to evaluate the contamination by this marine biotox ins of live bivalves intended for consumption, available on Polish market. The European Union has set some regulatory limits for PSP, DSP and ASP, i.e.: 800 µg/kg, 160 µg OA eq/kg and 20 mg/kg of meat. In the present study samples of bivalves were from different European countries. The following molluscs were examined: Atlantic razor clam (*Ensis directus*), blue mussel (*Mytilus edulis*), pacific oyster (*Crassostea gigas*), great scallop (*Pecten maximus*), japanese carpet shell (*Tapes semidecussatus*), dog cockle (*Glycymeris glycymerus*), hard clam (*Mercenaria mercenaria*) and cockles (*Cardium edule*). Live shellfish were collected from warehouses and markets and were transported to the laboratory at temperature 0 - 40C. The following tests were used for: PSP – Ridascreen Fast PSP S.C. (limit of detection 50 µg/kg), DSP - Okatest. Test for detection of Okadaic Acid-toxins group, Zeu-Immunotec (limit of detection 63 µg/kg) and ASP - ASP Elisa kit for quantitative determination of domoic acid, Biosense (limit of detection 0,01 mg/kg). In total, 207 samples of different molluscs were analysed. In 133 samples (64.3%) the presence of PSP biotoxins in the amount of 42.5–3327.6 µg/kg were detected. The legal limit was exceeded only in two samples of (2559.1 µg/kg and 3327.6 µg/kg). Biotoxins from the ASP group was detected in 18 samples (8.7%) of all samples. The legal limit for ASP was exceeding in 3 samples of scallops. The DSP group was identified in 43 samples (20.8%) of mussels in amounts ranging from 63 to 338 µg/kg. In two samples (oysters and japanese carpet shel l) the allowable value for DSP biotoxins was exceeded (338 µg/kg and 247 µg/kg. In the remain samples DSP toxin was not detected.

Keywords: *mussels, biotoxins, PSP, DSP, ASP*

J15
THE USE OF PERFORMANCE INDICATORS AS A TOOL TO EVALUATE THE QUALITY MANAGEMENT IN THE EU REFERENCE LABORATORIES: AN EXAMPLE OF THE DESIGN AND IMPLEMENTATION OF PERFORMANCE INDICATORS IN THE EU REFERENCE LABORATORY FOR MARINE BIOTOXINS

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European Union Reference Laboratories (EURLs) are designated by European Commission; being their legal functions described in the Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. One the key tasks of EURLs is to coordinate the network of National Reference Laboratories from EU Member States and to provide them with scientific support in all those aspects related with their own activity as National Reference Laboratories. EURLs are designated by the Council and the EU Commission which is in charge of assessing the performance of the EURLs. The novel approach to carry out this assessment is through Performance Indicators, which seem to be an adequate tool to perform such evaluation. The design and selection of relevant Performance indicators is highly dependent on the activity of the E URL, as well as the specific commodity. An important factor to be also taken into consideration is the number of Laboratories included in the Network. The selection of the objectives constitute the start point to establish the definition of the Performance Indicator, which should provide relevant information regarding the evaluated activity, the specific concept that should be measured must be clearly established and the calculation approach must be also indicated, as well as the establishment of responsibilities, defining threshold values when appropriate. Performance indicators should be further implemented and validated by comparison of the results achieved and expected. This study shows an example of design and application of a critical Performance Indicator in the European Union Reference Laboratory for Marine Biotoxins, based on the information above described. The results obtained show that a good design and implementation of Performance Indicators offer a very valuab le tool not only to evaluate the EURLs performance, but also facilitate the internal quality management of the EURL activities.

Keywords: *performance Indicators, quality management*

J16
THE SELECTION OF HPLC STATIONARY PHASE TO MINIMIZE MATRIX EFFECTS DURING LC–MS/MS ANALYSIS OF MULTIPLE MYCOTOXINS IN CORN

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LC-MS methods allow for rapid analysis of multiple analytes, increased selectivity and sensitivity. Recently a number of advancements have been made toward LC–MS analysis of mycotoxins in foods and feeds. This study investigated the use of C18 and Phenyl-hexyl HPLC column stationary phases for the analysis of twelve mycotoxin compounds. While C18 is a commonly used column, stronger retention of most analytes was observed on phenyl-hexyl stationary phase using the same gradient method. The separation of analytes from matrix is important, as often limited or no sample cleanup is applied during mycotoxin analysis, and matrix effects are highly probable. We will present the results of our investigation of matrix effects in corn meal using both C18 and phenyl-hexyl column chemistries, and discuss the recommended LC conditions for best overall method performance.

Keywords: *mycotoxins, food contamination, LC–MS, method optimization, phenyl-hexyl phase*

J17
EFFECT OF WHEAT HARVESTING AND CLEANING PROCESS ON LEVELS OF ALTERNARIA TOXINS

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In recent years, due to the climatic changes, mycotoxigenic fungi of the genus Alternaria have been recognised as important wheat contaminants. Only recently in vitro and in vivo studies on the toxicity of these mycotoxins demonstrated the high risks for animal and human health. On the other hand, scarce information is available on the behaviour of Alternaria toxins in food and feed during the processing. Therefore, the aim of this study was to investigate the possibilities of reduction of tenuazonic acid (TeA), alternariol (AOH) and alternariol monomethyl ether (AME) content during harvest (using laboratory thresher) and by use of wheat cleaning procedure (laboratory aspirator). Experiment was carried out on the wheat treated by fungicide and wheat inoculated by Alternaria tenuissima, while non treated wheat was used as a control. The content of Alternaria toxins in samples of wheat chaff, kernels without chaff, cleaned grain and impurities were analyzed by HPLC with electrospray ionization triple quadrupole mass spectrometry (LC–ESI–MS/MS). The content of Alternaria toxins in samples of wheat chaff and kernels without chaff point out at higher content in chaff than in kernels without chaff. Namely, in the chaff of treated and non treated wheat samples all three examined Alternaria toxins were detected, while in chaff of protected wheat sample AOH was not detected. Also, in samples of wheat kernels without chaff the highest concentration of TeA was detected in inoculated wheat kernels. The detected levels of TeA were about three times higher in chaff compared to kernels from all treatments. The concentration of AOH was doubled, while the detected level of AME was about three times higher in chaff of inoculated wheat sample compared to chaff of non treated wheat sample. AOH was not detected in wheat samples without chaff in any of analyzed cases, while AME was only detected in inoculated wheat samples without chaff. The cleanin g of wheat grain in the control, fungicide protected and inoculated wheat samples led to the reduction of the levels of TeA of 60.5%, 59.3%, and 46.4% in comparison to the initial TeA concentrations in the unprocessed wheat samples. In the impurities removed from the examined samples, there was an increase in TeA content by 6.2, 9.5, and 4.7 times in comparison to the initial TeA concentration in the unprocessed wheat samples. Regardless of the treatment used, AOH and AME were only quantified in the removed impurities, while from dibenzopyrone derivatives only AME was quantified in the unprocessed inoculated wheat sample. AME concentration in the impurities removed from inoculated wheat was 3.9 times higher in comparison to the initial concentration in unprocessed wheat. The presented observations point to the possibility that during harvest and cleaning of wheat reduction of the content of Alternaria toxin by about 50% in comparison to its initial concentration in the unpro cessed wheat samples may be achieved.

Keywords: *alternaria toxins, LC–MS/MS, wheat, harvesting, cleaning*
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J18
DISTRIBUTION OF AFLATOXINS AND ASPERGILLUS FLAVUS IN STORED MAIZE

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Aflatoxins (AFs) are highly toxic secondary metabolites produced primarily by the fungi Aspergillus (A.) flavus. AFs and A. flavus are very often found as contaminants of maize, especially in tropical and subtropical regions. Even though Republic of Serbia is located in the continental climate belt, maize growing season 2012 was characterized as extremely hot and such weather conditions influenced presence of AFs in approximately 75% of maize. The aim of this study was to investigate distribution of A. flavus and AFs in naturally contaminated stored maize. For this purpose, 16 maize samples were taken from different places in two warehouses and analyzed. A. flavus and AFs were found in every of eight samples taken from the first warehouse with colony number from 6.20 to 57.5 × 103 cfu/g and concentration range from 10.4 to 88.1 μg/kg, respectively. Every analyzed samples from the second warehouse also contained A. flavus (colony number from 6.10 to 105 × 103 cfu/g) while two out of eight samples were not contaminated with AFs. However, the remaining six samples contained AFs in wide concentration range from 16.5 to 183 μg/kg. Obtained results indicate an uneven distribution of A. flavus and AFs in both examined warehouses with naturally contaminated stored maize. Furthermore, AFs and A. flavus were found in a wide range of concentration as well as colony number. In general, samples with highest number of A. flavus colonies were also contaminated with high AFs concentrations. It is necessary to emphasize that due to the uneven distribution of A. flavus and AFs in stored maize it is necessary to direct particular attention to sampling in order to obtain reliable concentrations of AFs.

Keywords: *stored maize, Aspergillus flavus, aflatoxins, distribution*

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J19
THE DETERMINATION OF THE MYCOTOXIN CONTENT IN DISTILLER'S DRIED GRAIN WITH SOLUBLES (DDGS) USING A MULTI-ANALYTE UHPLC–MS/MS METHOD

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There are more than 300 potential mycotoxins that can contaminate food and feed and cause adverse effects in humans and animals. In the European Union there are maximum limits for aflatoxin B1 and guidance values for deoxynivalenol, zearalenone, ochratoxin A, fumonisin B1 and B2 for determining the acceptability of the cereal products to be used as animal feed. In the recent years dried distiller's grain with solubles (DDGS) - a co-product of alcohol fermentation (beverage or biofuel production) has become an alternative type of animal feed ingredient. It appears a good choice as a feed material due to its high content of proteins, fats and fibres. However, there is a paucity of data on the safety of DDGS with regard to mycotoxin contamination. Thus a UHPLC–MS/MS method was developed and validated for the detection and quantification of 77 mycotoxins or other fungal metabolites in animal feed ingredients. The method was used to analyze 169 DDGS samples produced from wheat, maize and barley and 61 grain samples collected from the European biofuel manufacturing and feed industry. This study provides the first comprehensive dataset on the co-occurrence and levels of mycotoxins in different DDGS types. All 52 maize DDGS samples contained regulated mycotoxins fumonisins B1 and B2 and the contamination ranged from 81.0 to 6890 µg/kg for fumonisin B1. The enniatins and beauvericin mixtures were also present in every sample analyzed with highest levels of beauvericin (46.1 to 561 µg/kg). Additionally, a substantial number of co-occurring mycotoxins such as fusaric acid (present in 100% of the samples), equisetin (98%), mycophenolic acid (96%), meleagrin (81%), T-2 toxin (81%), aflatoxin B1 (75%) and deoxynivalenol (62%) were identified. All 99 wheat DDGS samples were contaminated with regulated mycotoxin deoxynivalenol (from 39.3 to 1120 µg/kg), a mixture of 6 to 12 ergot alkaloids (with total ergot alkaloids content from 4.7 to 12 30 µg/kg) and a mixture of enniatins with highest level of enniatin B (from 164 to 1490 µg/kg). Mycophenolic acid was also present in 98% of the wheat DDGS samples. Nine barley DDGS samples were contaminated with a mixture of deoxynivalenol, fumonisins, zearalenone, aurofusarin, enniatins and beauvericin, equisetin, fusaric acid, T-2/HT-2 toxins, ochratoxin A, meleagrin, mycophenolic acid and ergot alkaloids. A comparison of the mycotoxin content in grains and the DDGSs that were produced from them revealed higher content of mycotoxins in the DDGS samples. The data generated in this study points to potential issues with the use of DDGS in terms of affecting the performance and health of the recipient animals. Routine screening for mycotoxins in DDGS is highly recommended to allow the highlighted risks to be effectively managed.

Keywords: *mycotoxins, distiller's dried grain with solubles, grain, multi-mycotoxins contamination, animal health*

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J20
MULTI-TOXIN ANALYSIS USING IMMUNOAFFINITY COLUMN CLEAN-UP FOR A RANGE OF SAMPLES PRIOR TO LC–MS/MS DETECTION

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Frequently in mycotoxin analysis there is a need to analyse for combinations of mycotoxins as dictated by the susceptibility of the commodity to specific fungal infection and the regulatory limits which apply. Most official methods, which have been rigorously validated, stipulate the use of immunoaffinity column clean-up prior to HPLC analysis. R-Biopharm Rhone's immunoaffinity columns offer a versatile solution for multi mycotoxin analysis whereby the immunoaffinity columns can be used in tandem with one another to cover the regulated mycotoxins applicable to a particular food matrix. AOF MS–PREP® and DZT MS-PREP® immunoaffinity columns were tested in tandem to determine the applicable mycotoxins (total aflatoxin, ochratoxin A, fumonisin, deoxynivalenol, zearalenone, T-2 and HT-2) in a number of cereals and cereal based products. The samples were analysed using a single extraction followed by immunoaffinity clean-up with the columns connected in tandem prior to LC–MS/MS detection. Matrices analysed were maize based infant food, beer, bread and breakfast cereal. When testing the various samples using the tandem method results met EU Method Performance Criteria in terms of recovery and % RSDr. This study shows that a single extraction with immunoaffinity column clean-up can be utilised for the analysis of 11 mycotoxins in one LC–MS/MS run. This approach is not only cost effective but offers a greater flexibility of testing options to customers analysing for a wide range of mycotoxins.

Keywords: *multi-toxin, LC–MS/MS, immunoaffinity*

J21
VALIDATION OF A METHOD FOR THE ANALYSIS OF CITRININ IN CEREALS USING IMMUNOAFFINITY COLUMNS

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Citrinin is produced by a number of Aspergillus and Penicillium fungi and has been found in a variety of foods such as grains, cheese and red yeast rice, the latter of which is regulated in the EU (Commission Regulation (EU) No 212/2014). Although legislation is currently only in place for red yeast rice there is considerable interest in Europe with regards to levels in foods including cereals, as this toxin is considered as a potential issue during storage often occurring simultaneously with ochratoxin A with both toxins considered as potential agents of Balkan endemic nephropathy. R-Biopharm Rhône has developed a new immunoaffinity column which selectively isolates and concentrates citrinin from a wide range of commodities including cereals and red yeast rice. A simple extraction with 75% methanol was used to analyse a range of cereal samples spiked at 100 ppb. Average recoveries ranged from 74% for multi-grain cereal to 96% for oat groats demonstrating that the new EASI-EXTRACT® CITRININ columns were suitable for the clean-up of the toxin from a wide variety of cereal samples resulting in improved chromatography and lower limits of detection.

Keywords: *citrinin, immunoaffinity, clean-up*

J22
QUANTITATION OF 28 PYRROLIZIDINE ALKALOIDS IN COMMERCIAL HERBAL TEAS, ESPECIALLY ROOIBOS TEA USING UHPLC/MS/MS – IS THE ROOIBOS TEA STILL BE SAVED?

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Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by more than 6000 plant species form the families Asteraceae, Boraginaceae, and Fabaceae as protection against herbivores. In Europe most PA containing plants occur in the Mediterranean but due to climate change a further spread is expected. PAs are causing hepatotoxic, mutagenic or carcinogenic effects. Food contaminations have been reported for honey containing nectar from composite plants and for herbal teas if weeds were accidentally mixed with crops. In July 2013 the Federal Institute for Risk Assessment (BfR) issued a call for action to improve data regarding the occurrence of PAs in herbal teas. We show a recently validated method for the quantitation of 28 PAs by UHPLC/MS/MS and its application to the analysis of honey and herbal teas. Extraction of honey and herbal teas was done with acidified water. Crude extracts were cleaned up using a strong cation exchange SPE. The refined extract was evaporated to dryness, reconstituted with mobile phase and injected into the UHPLC/MS/MS system. Chromatography was performed under gradient conditions with acidified ammonium formate and methanol as mobile phases using a C18 2.1 × 100 mm, 2.7 µm core shell particle column. Detection of most currently commercially available pyrrolizidine alkaloids and their N-oxides was performed using a highly sensitive dual ion funnel triple quadrupole MS operated in dynamic MRM in positive mode. Three MRM transitions have been acquired per compound as especially in the plant material matrix interferences showed up on one and sometimes even on two MRM transitions. The UHPLC separation provides improved chromatographic resolution of isomeric PAs; e.g. it allows for the baseline separation of intermedine and lycopsamine. An overview quantitation was based on matrix matched calibrations with a mixed matrix of herbal teas to compensate for matrix effects in the electrospray ionization. The exact quantitation was realized with a standard addition of the sample. For validation honey and tea samples were spiked with the PAs before extraction at different concentrations and were analyzed with the described method. Limits of quantification (LOQs) were in the sub or low µg/kg range for the evaluated PAs in the honey and slightly higher in the herbal teas as the recovery of the N-oxides was negatively affected by the additional clean-up steps. Challenging for the routing analysis is also the sampling, because of the inhomogeneous distribution of the PAs in the sample. When applying the method to rooibos tea samples purchased from a local market, contaminations with PAs in the range from 143 µg/kg to 2305 µg/kg were observed. The analysis of 28 PAs in 24 samples demonstrate that PAs belonging to the senecionine-type (retrorsine, senecionine, seneciphilline and there N-oxides) were most frequently found. Because of their structure (macrocyclic diesters) these PAs have the highest toxicity.

Keywords: *pyrrolizidine alkaloids, LC–MS/MS, rooibos tea*

J23
ASSESSMENT OF MIXTURES OF MYCOTOXINS IN CEREAL BASED FOODS AVAILABLE IN PORTUGUESE MARKET

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Mycotoxins are secondary metabolites of fungi that cause toxic and carcinogenic outcomes in humans exposed to them [1]. Mycotoxins affect several commodities including cereal grains and their finished products, infant formula and baby foods [2]. This study aimed to determine the incidence and levels of 20 mycotoxins and metabolites (AFB1, AFB2, AFG1, AFG2, AFM1, OTA, NIV, NEO, DAS, FUS-X, DON, 15-AC-DON, 3-AC-DON, HT-2, T-2, VER, T-2 TETROL, T-2 TRIOL), in breakfast cereals and cereal based baby foods available in the Portuguese market, and compare the results with the maximum limits established by the EU. Breakfast cereal samples (n=26), including corn, wheat, oat, rice and multigrain, and twenty cereal based baby foods (n=20) were collected from supermarkets in Lisbon region and analyzed by HPLC–FLD, LC–MS/MS and GC–MS. Results showed that 88 % breakfast cereals samples and 50% of cereal based baby foods were contaminated with mycotoxins (with values above the detection limit), although all samples presented levels below the maximum limits established by the Commission Regulation 1881/2006 [3]. Regarding breakfast cereals samples, OTA and DON were the most commonly detected mycotoxins, with 88% and 73% of samples revealing values above the LOD, respectively. The co-occurrence of different mycotoxins in the same sample was observed in 92% of the analyzed samples. From these, 46% include mixtures of 3 or 4 mycotoxins. Regarding cereal based baby foods, OTA and AFM1 were the most commonly detected mycotoxins with 50% and 40% of samples revealing values above the LOD. The co-occurrence of mycotoxins was observed in 35% of the analyzed samples. These results are accordingly to those reported by Juan et al (2014) [4] and Iqbal et al (2014) [5]. These results contribute to the increased knowledge on mycotoxin contents in cereal based foods marketed in Portugal, and they highlight the deep need of further studies to overcome the absence of legislated limits for mycotoxins in breakfast cereals other than DON and FB1 and the absence of legislated limits for mycotoxin mixtures in food. The last issue is particularly important considering the potential synergistic effects that could occur between mycotoxins and its potential impact on human and, mainly, children health.

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Keywords: *cereal based foods, mycotoxins, chemical mixtures, Portugal*

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J24
DETECTION OF AFLATOXIGENIC FUNGI AND TOXINS FROM EXPORT GRADE PEANUT SAMPLES: COMPARISON OF QPCR AND LC/MS TECHNIQUES

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Aflatoxins are toxic secondary metabolites produced by various species of Aspergillus fungi and have gained global significance as a result of their deleterious effects on human and animal health. In this study, we detected both the toxin pathway gene, O-Methyltransferase (omt-1), of A. flavus as well as the toxin, aflatoxin B1, produced on peanut seeds by using probe-based qPCR and LC/MS assays, respectively, by conducting a time-course experiment. The peanut samples were artificially infected with fungal spores, and sampling was performed at different time points. The results revealed that the aflatoxin amount was well within the minimum residue level limit (MRL) specified by EU regulation up to Day 1 after fungal inoculation, but exceeded the MRL from Day 2 onwards. Similarly, the quantification cycles (Cq) for the omt-1 gene also decreased in a time-dependent manner, indicating the fungal growth on peanut. A time-dependent increase of DNA copies and the amount of secondary metabolite was evident. These results suggest that combination of the qPCR and LC/MS methods provide confidence for critical evaluation of peanut consignments.

Keywords: *peanut, aflatoxin, detection, probe based qPCR, LCMS*

J25
DEFINING FITNESS-FOR-PURPOSE FROM ERGOT ALKALOIDS PROFICIENCY TESTS

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Proficiency tests for mycotoxins are well established by many providers. Indeed, the methods are so well characterised that their fitness for purpose is automatically defined by the Horwitz equation (original or Thompson-modified forms). This holds true for aflatoxins, patulin, fumonisins, trichothecenes, and ochratoxin A across a wide variety of matrices. A number of laboratories are now offering related testing services for ergot alkaloids and FAPAS has started to provide proficiency tests to support their quality assurance. The results from the first two proficiency tests, however, have been widespread. The first proficiency test at low levels was unable to assess 3 of the 12 analytes and the uncertainty was high for another 6 analytes. The second proficiency test at much higher concentrations was made more successful by adopting the 22% modified Horwitz equation, rather than the original Horwitz equation. This suggests that ergot alkaloids analysi s is not nearly as straightforward as for the established mycotoxins methods. In the absence of any characterising interlaboratory trials, these initial two proficiency tests indicate that fitness-for-purpose of ergot alkaloids analysis must be generous.

Keywords: *FAPAS, proficiency test, ergot alkaloids*

J26
IN SITU ANALYSIS OF TRICHODIENE AS A VOLATILE BIOMARKER FOR THE FAST AND NON-INVASIVE GAS-PHASE ANALYSIS OF CEREAL MYCOTOXINS

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Fungal infection of cereals such as wheat, barley and corn is an increasingly grave nutritional problem in many countries worldwide. Producing a wide range of biologically active secondary metabolites (trichothecenes, fumonisins and zearalenone), the genus Fusarium belongs to the most harmful and devasting plant pathogens with Fusarium head blight (FHB) being the most economically relevant diseases of wheat. Besides causing strong economic losses, these so-called Fusarium mycotoxins also pose a potential risk to human and animal health and are, therefore, of particular importance to agriculture and food safety. Typically, mycotoxigenic moulds and consequently small percentages of extremely contaminated portions ("hot spots") are randomly distributed in a lot. Therefore, an effective sampling procedure for cereal mycotoxin detection or quantification represents a complex challenge for operators involving invasive and cost intensive steps. During studies for microbial volatile organic compounds (MVOCs) indicating an infection with *Fusarium*, trichodiene was identified as a unique biomarker for one of the largest groups of the mycotoxin family. The sesquiterpene trichodiene constitutes the only volatile biosynthesis intermediate of the common trichothecenes. Up to now, there is no commercial calibration standard available enabling a qualitative and quantitative analysis of trichodiene. The project work focusses on the development of a fast, easy-to-handle and non-invasive gas-phase detection of trichodiene in the field. Therefore, the compound was prepared by total synthesis based on a tandem orthoester Claisen rearrangement – oxidation – Robinson annulation strategy providing the racemic natural product in 9 steps and 8% overall yield. Its structure was fully elucidated by NMR and MS. With the reference standard in hand, a protocol was established for the headspace analysis of trichodiene above crop spikes by GC/MS in the < 100 µg/kg range. Besides a sample survey and a trichodiene-trichothecene correlation study, it is aimed to transfer the validated analytical method from the laboratory into a field-portable analytical system. Trichodiene is the first and only volatile biogenetic precursor of *Fusarium* mycotoxins and its early and fast detection *in situ* might therefore be of potential interest as a marker for a *Fusarium* infestation and thus food safety analysis.

Keywords: *trichodiene, fusarium mycotoxins, fungal volatiles, synthesis, headspace analysis*

J27
MYCOTEST − DEVELOPMENT OF FAST ANTIBODY-BASED SCREENING TESTS FOR THE DETECTION OF HARMFUL MYCOTOXINS IN FOOD AND FEED

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The contamination of food and feed with harmful fungal derived mycotoxins is a problem worldwide. Over 400 different potential mycotoxins are now known, some of them have been studied in great detail, while the data about toxicity and occurrence for many others are scarce. This information is required to determine the risk in relation to the animal and human health and establish maximum permitted levels that do not pose any health concerns. The fast elimination of contaminated food and feed from the supply chain is crucial for the protection of human and animal health as many of these toxins can cause serious diseases both after acute and chronic exposure. The occurrence data cannot be provided without fast and reliable detection methods that allow quantification of these toxic contaminants. This research, therefore, is focused on delivering new fast antibody-based (ELISA) screening methods for the detection of three important mycotoxins: T-2/HT-2 toxins, oc hratoxin A (OTA) and sterigmatocystin (STE) and on the development of a first multiplex flow-through rapid (FTR) test for the simultaneous detection of mycotoxins in food and feed. T-2/HT-2 belong to the trichothecenes group of mycotoxins produced by Fusarium ssp. that can contaminate a wide range of agricultural crops. Their toxic effects include inhibition of protein synthesis and alteration of cell membrane functions. There are currently indicative levels in food (between 15 and 1000 µg/kg) for these toxins in the EU and the repetitive findings above these levels trigger investigations into the reasons for contamination. OTA is produced by fungi from Aspergillus and Penicillium and it is a potent renal toxin, immunotoxic, neurotoxic and teratogenic. The maximum levels of OTA in cereals, dried vine fruit, coffee, wine, grape juice and baby food have been set to 0.5–10 µg/kg in the EU. Aflatoxins (B1, B2, G1, G2 and M1) are a group of the most potent carcinogens found in nature and STE is one of the precursors in aflatoxin biosynthesis. Also STE has been linked to genotoxicity and carcinogenicity. The regulation in the EU requires many types of foods such as cereals, nuts and milk to be tested for aflatoxins, but not for STE, therefore little is known about its occurrence. The tests developed in this project could be used by food and feed producers, importers and testing laboratories to assure food and feed safety on the European market. The initial characterization of the available antibodies for T-2/HT-2 toxins and OTA has been complete. The best two antibodies will be selected for the development of simple ELISAs. The next stage of the project will include production of a specific antibody to sterigmatocystin and selecting of the available antibodies for the regulated mycotoxins for the development of a multiplex test.

Keywords: *mycotoxins, food, fungal contamination, ELISA, screening*

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J28
FAST AND SENSITIVE AFLATOXIN B1 AND TOTAL AFLATOXINS ELISA DEVELOPMENT, VALIDATION AND APPLICATION FOR PEANUT MONITORING

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Aflatoxins (aflatoxin B1, B2, G1 and G2) produced by toxigenic fungi can contaminate different agricultural commodities such as corn and peanuts. Due to their toxic effects in humans and animals fast and validated method for the detection of aflatoxins in food and feed are required for the identification of the contaminated batches before they are processed into final products and placed on the market. In order to address the need for better and improved detection methods seven monoclonal antibodies for aflatoxins with a good compromise between sensitivity and cross-reactivity were produced. Two best antibodies showing IC50 of 0.037 ng/mL and 0.031 ng/mL for AFB1 were applied in simple and fast direct competitive ELISA tests for the detection of aflatoxin B1 and total aflatoxins. The developed ELISA kits were validated for peanut matrix. The detection capabilities of aflatoxin B1 and total aflatoxins ELISAs were 0.4 µg/kg and 0.3 µg/kg for aflatoxin B1, re spectively, which are one of the lowest reported values. A critical assessment of the performance of the total aflatoxin ELISA kit for the detection of aflatoxins B2, G1, and G2 was also performed. The kits were used to analyze 32 peanut and peanut butter samples purchased locally and two samples containing small but detectable amount of aflatoxin B1 were identified, what was further confirmed by LC–MS/MS analysis. The developed ELISA kits were also tested in Proficiency Test and were demonstrated to have an excellent accuracy. These kits will be transformed into commercial products for the protection of consumer in the EU and worldwide.

Keywords: *aflatoxins, mycotoxins, immunoassay, food, peanuts*

J29
OCCURRENCE OF DIARRHETIC SHELLFISH POISONING TOXINS IN MARINE MOLLUSCS FROM NORTH-EAST OF ITALY

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Italy is the third European producer of marine molluscs and the North Eastern part of the Country is a leader in the national shellfish industry. While hydrophilic biotoxins as domoic acid and saxitoxin have never been detected so far in this area, the phenomenon of lipophilic toxins accumulation in shellfish has become more and more frequent in the recent years. Lipophilic toxins were classified into four groups: okadaic acid (OA), including dinophysistoxins (DTXs); pectenotoxins (PTXs), azaspiracids (AZAs) and yessotoxins (YTXs).
The consumption of shellfish contaminated with lipophilic marine biotoxins, produced by toxic dinoflagellates, causes a severe gastrointestinal illness known as Diarrhetic shellfish poisoning (DSP).
To protect public health European Union established a series of regulations for the control of lipophilic toxins. Thus, Regulation (EC) Nº 853/2004 lays down the maximum levels for lipophilic toxins in bivalve molluscs before being placed on the market for human consumption: for OA, DTXs and PTXs together, 160 micrograms of OA equivalents per kilogram; for AZAs, 160 micrograms of AZA equivalents per kilogram. The Regulation (EU) No 786/2013 fixed the permitted limits of YTXs in live bivalve molluscs to 3.75 milligrams of YTXs equivalent per kilogram. Regarding methodologies, the Commission Regulation (EU) No 15/2011, established the EU-RL LC-MS/MS method as the reference method for the detection of lipophilic toxins for the purposes of official controls at any stage of the food chain. On this basis the Chemical department of Istituto Zooprofilattico Sperimentale delle Venezie has developed and accreditated (according to ISO 17025:2005) a LC-MS/MS screening/confirmatory method since July 2012.
In this work, results of the analysis of more than 3000 samples of shellfish tissue, coming from the North East of Italy, in the period 2012–2014 are presented. Samples analysed consisted in mussels (*M. galloprovincialis*, 80%), clams (*R. philippinarum, C. gallina,* 13%) and other species (*C. chione, Chlamys spp, C. gigas* etc, 7%).
The most frequently lipophilic toxins detected over the Limit of Quantitation were OA and YTXs in the *M. Galloprovincialis* samples. The not compliant samples percentage did not exceed 2% for both and, in general, they resulted more frequent during late summer and autumn.

Keywords: *lipophilic toxins, diarrhetic shellfish poisoning (DSP), LC–MS/MS*

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J30
MONO- AND DIACETOXYSCIRPENOL – DETERMINATION IN CEREALS AND CEREAL-BASED FOODS

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The contamination of food and feed with moulds and their secondary metabolites has become a global issue with a significant health-related and economic impact. Due to the serious toxic effects exerted by mycotoxins, the surveillance and reduction of these compounds are two of the most relevant challenges concerning global food and feed safety. Trichothecenes are one of the major classes of mycotoxins, mainly produced by moulds belonging to Fusarium species, especially F. sporotrichioides, F. poae and F. equiseti. This mycotoxin family can be divided into four subgroups (types A–D), according to their chemical structure. The major type A trichothecenes such as 15-monoacetoxyscirpenol (MAS), 4,15-diacetoxyscirpenol (DAS), T-2 toxin (T-2) and HT-2 toxin (HT-2) are in general more toxic than type B trichothecenes. There are currently no legal limits for type A trichothecenes in food, however, within the European Commission respective discussions are ongoing for T-2 and HT-2. The aim of the presented study was to verify whether the official German method for the determination of T-2 and HT-2 in oats and oat products [1] may be adapted to the detection of MAS and DAS. Therefore, the steps of extraction and cleanup were optimized. The highest absolute recoveries (MAS: 89.1%, DAS: 92.3%) were obtained for acetonitrile/water (80:20, v/v) as extraction solvent in combination with the use of an Ultraturrax Tube Drive Control homogenizer and subsequent cleanup based on solid phase extraction (activated carbon/alumina/ diatomaceous earth; 7:5:3; *w/w/w*). Limits of detection and quantification were lower than 0.03 µg/kg and 0.11 µg/kg, respectively. The optimized method was applied to the analysis of 11 commercial products (cereals and cereal-based foods) obtained from local supermarkets. All samples contained the mycotoxins T-2 and HT-2 in contents of 0.06–6.3 and 0.27–19.0 µg/kg, respectively. Nine samples contained MAS (0.19–0.79 µg/kg), whereas only seven samples were found to have DAS contamination (0.06–0.22 µg/kg).

[1] Technical rule BVL L 15.04-1:2012–01. Standard method of the German federal office of consumer protection and food safety for the determination of T-2 toxin and HT-2 toxin in oats and oat products by HPLC–MS/MS after solid phase cleanup.

Keywords: *fusarium toxins, trichothecenes, food analysis, SIDA-HPLC–MS/MS, multitoxin extraction*

J31
USING ION MOBILITY MASS SPECTROMETRY AND COLLISION CROSS SECTION AREAS TO ELUCIDATE THE A AND B EPIMERIC FORMS OF GLYCOSLATED T-2 AND HT-2 TOXINS

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Mycotoxins are secondary fungal metabolites, toxic to human and animals. Toxigenic fungi often grow on edible plants, thus contaminating food and feed. Plants can alter the chemical structure of mycotoxins as part of their defence against xenobiotics. The extractable conjugated or non-extractable bound mycotoxins formed remain present in the plant tissue but are currently neither routinely screened for in food nor regulated by legislation, thus they may be considered “masked”. Fusarium species mycotoxins (deoxynivalenol, zearalenone, fumonisins, nivalenol, fusarenon-X, T-2 toxin, HT-2 toxin, fusaric acid) are prone to metabolism or binding by plants. Toxicological data are scarce, but several studies highlight the potential threat to consumer safety from these substances1. In particular, the possible hydrolysis of masked mycotoxins back to their toxic parents during mammalian digestion raises concerns. Masked mycotoxins may elude conventional analysis be cause of changed physicochemical properties of their molecules leading to modified chromatographic behaviour, or due to modification of an epitope recognised by antibodies used for the detection, or because of impaired extraction efficiency caused by increased polarity when a less polar solvent is used for the extraction of non-modified mycotoxins. All of these effects may lead to a potential underestimation or overestimation of the total mycotoxin content of the sample. In this study we report the use of High Definition Mass Spectrometry (HDMS) as a powerful tool for the separation and characterisation of the α and β epimeric forms of synthetic glycoslated T-2 and HT-2 toxins. High Definition mass spectrometry is a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Ion mobility spectrometry (IMS) is a rapid orthogonal gas separation phase technique which allows another dimension of separation to be obtained within an Ultra Performance Liquid Chromatography (UPLC) timeframe. Compounds can be differentiated based on their size, shape and charge. In addition, both precursor ion and fragment ion information can be simultaneously acquired in a single injection in an HDMS experiment, referred to as HDMSE. HDMS data not only provides additional peak capacity but also insights into the molecular characteristics of the analytes for example, the elucidation of different isomeric spec ies and intra-molecular sites of protonation. This additional information can been used to inform and optimise the analytical strategy adopted. The ion mobility data generated has been used to calculate the collision cross section area (CSS) values within the data processing software (UNIFI v.1.8) for the target mycotoxins. The combination of CCS value, retention time, exact mass and fragmentation information provides unique and unequivocal characteristic signature of the glycosylated toxins.

Keywords: *masked mycotoxins, ion mobility, mass spectrometry, CCS*

J32
GLOBAL MYCOTOXIN SURVEY 2015 FOR ANIMAL FEED

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Since 2005, BIOMIN has been conducting an annual mycotoxin survey, monitoring the incidences of different mycotoxins in several agricultural commodities intended for use in animal feed. The samples are collected on a worldwide basis and are analyzed for the following mycotoxins: Afla (aflatoxins B1, B2, G1, G2), ZEN (zearalenone), DON (deoxynivalenol), T-2 toxin, FUM (fumonisins B1 and B2) and OTA (ochratoxin A). Analysis is carried out in different laboratories, using mainly HPLC but also LC–MS/MS and ELISA methods. In the first two quarters of 2015, more than 3200 samples were analyzed. As expected, regional differences can be observed. For example, the most prevalent mycotoxin in Europe appears to be DON: 83% of > 1500 tested samples were positive, i.e. showed values above the limit of quantitation of 50 ppb (HPLC) or 1 ppb (HPLC–MS/MS). The average level of DON in the positive samples is 1727 ppb. On the other hand, e.g. in South America, DON is found in only 25% of all samples (on average 661 ppb), but 61% of all tested samples are contaminated with FUM, with an average value of 1156 ppb. In general, 12% of all samples were tested negative, 23% of samples were contaminated by a single mycotoxin and the remaining 65% of samples were positive for more than one of the tested mycotoxins. From these data it is obvious that mycotoxins are a worldwide threat and that further research on the effects of co-occurrence of several mycotoxins is warranted because co-contamination proves to be the rule, not the exception.

Keywords: *mycotoxin, occurence, feed*

J33
PRESENCE OF PATULIN IN FRUIT JUICES AND EXPOSURE OF POPULATION IN SERBIA

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This study was conducted to evaluate the presence of patulin, a toxic secondary metabolite of the fungi belonging to the *Penicillium, Aspergillus* and *Bysochlamys* species, in fruit juices made in Serbia and to estimate corresponding population exposure.

In total 121 samples of fruit juices made of apple (20) or apple and other fruit (69), with a volume size from 0.75 to 2 L, and juices with straw (32; 0.2 L) were collected from the market in 2014 and 2015, in original packages, as available to the consumers in the city of Novi Sad. Analytical determination of patulin was carried-out by high performance liquid chromatography with ultra-violet detection. Laboratory method performance was characterized with limit of quantification of 1 ng/g, recovery of 85% and 70% for clear and cloudy juices, respectively, precision in terms of relative standard deviation <5%. Results were corrected for in-house recovery.

The study revealed an overall incidence of patulin occurrence in juices of 36%, with mean patulin level at 2.5 ng/g. Frequency distribution showed that in 29.2% of the samples patulin concentration ranged from 1 to 10 ng/g, 4.5% between 10 and 25 ng/g, 2.3% above 25 but less than 50 ng/g, whereas none of the samples exceeded the maximum allowable level of 50 ng/g. Comparison of pure and mixed apple juices showed 2.3-fold higher incidence (65% *vs* 28%) and 2-fold higher mean patulin level (4.0 *vs* 2.1 ng/g) in pure apple juices. Higher mean patulin concentrations were also observed in juices with 50% fruit content when compared with 100% ones. Regarding juices with straw, overall patulin incidence was 44%, with 41% of the samples showing patulin contamination below 10 ng/g and only one sample above (23.8 ng/g). Mean patulin concentration in pure apple juices was 3.1 ng/g, whereas mixed juices showed 3-fold lower contamination, giving overall mean of 1.7 ng/g in juices with straw.

The exposure of the adult population was assessed by combining fruit juice consumption data taken from the official Serbian national consumption survey (0.05 kg/person/day) with the mean content of patulin recorded in the present study, and compared with the provisional maximum tolerable daily intake (PMTDI) of patulin (400 ng/kg body weight/day). Estimated daily intake of patulin for an adult represented less than 1% of PMTDI. In case of children, the major consumers of juice in pack with straw, higher consumption of fruit juices is expected (specific data not provided). Taking into account body weight of 20 kg and volume of one juice package (0.2 L), patulin intake was estimated to be around 4% of PMTDI.

To conclude, high incidence but low contamination level resulted with low exposure, so that adverse health effects caused by patulin intake in consumers of fruit juices in Serbia are not to be expected.

Keywords: *patulin, fruit juice, HPLC*

J34
DEVELOPMENT OF A MULTI-RESIDUE METHOD FOR THE ANALYSIS OF MYCOTOXINS, INCLUDING MASKED MYCOTOXINS, IN CEREAL-BASED FOOD BY UHPLC–MS/MS

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Mycotoxins are toxic secondary metabolites produced by fungi that readily colonise crops in the field or during storage; these compounds represent a serious problem for public health and may cause significant economic losses. The aim of this research is develop and validate multi-residue test methods for mycotoxins and their masked metabolites in cereal-based food and beverages using ultra high performance liquid chromatography coupled to tandem mass spectrometry. In the first part of this work, both chromatography expedients and mass spectrometry conditions were optimised in order to achieve the best separation, ionisation and fragmentation for each analyte. A sample preparation procedure was developed based on the QuEChERS methodology. The impact of many types of salts, (ammonium sulphate, sodium sulphate, magnesium sulphate, sodium chloride), along with different combinations of them in varying amounts, was evaluated. Also, extraction solutions at differe nt pH were tested to obtain the best overall recoveries for all the analytes. Subsequently, the developed technique was successfully applied to the analysis of mycotoxins in wheat samples, including all the regulated mycotoxins, the emerging mycotoxins enniatins and the masked metabolites deoxynivalenol-3-glucoside (D3G) and T-2-glucoside (T2G). The limit of quantifications (LOQs) ranged from 0.5 µg/kg to 100 µg/kg, depending on the sensitivity of each analyte.

Keywords: *mycotoxins, masked mycotoxins, cereals, UHPLC–MS/MS, QuEChERS*

J35
DETECTION OF PARALYTIC SHELLFISH POISONING TOXINS AND DOMOIC ACID BY UHPLC–MS/MS

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Domoic acid is a marine biotoxin causing amnesic shellfish poisoning (ASP) in humans; this water-soluble cyclic amino acid is mainly produced by marine red algae of the genus Chondria and diatoms of the genus Pseudo-nitschia. Paralytic shelfish poisoning toxins are neurotoxins biosynthesized by marine dinoflagellates (Alexandrium, Gymnodinium and Pyrodinium genus) and some freshwater Cyanobacteria. Bivalves and shellfish accumulate phycotoxins during filter feeding and their consumption could therefore lead to human poisoning. The CEN/TC 275 reference method for the domoic acid group analysis is based on high-performance liquid chromatography-UV in Europe. On the other hand, the so-called Laurence method has replaced the AOAC mouse bioassay for routine analysis of PSPs in shellfish. This method, based on pre-column oxidation (Pre-COX) and HPLC-fluorescence detection, suffers from several drawbacks among which its inability to separate epimeric pairs and its complexity (multiple SPE clean-up, multiple oxidation steps, interpretation of chromatograms requesting harsh training of operators to avoid confusion between PSP multiple peaks confusion) and related long analysis delay. The aim of this work was to develop an UHPLS–MS/MS method enabling a fast screening of shellfish routine samples for both ASP and PSPs. The method includes a solid/liquid extraction and an UHPLC analysis of the toxins in MRM mode. The targeted compounds are domoic acid (DA), saxitoxin (STX), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX), decarbamoylneosaxitoxin (dcNEO), gonyautoxins 1-6 (GTX 1-6), N-sulfocarbamoyl gonyautoxins 1-4 (C1-4) and decarbamoylgonyautoxin 2-3 (dcGTX2&3). The method was successfully validated according to Decision 2002/657/CE and tested with materials from 2013, 2014 and 2015 EURL-MB Proficiency Tests.

Keywords: *PSPs, domoic acid, shellfish, UHPLC–MS/MS*

J36
OCCURRENCE OF ERGOT ALKALOIDS IN FEED AND RYE SCLEROTIA IN THE CZECH REPUBLIC

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Ergot alkaloids (EAs) are mycotoxins produced predominantly by fungi of the Claviceps genus, which infect more than 400 plant species including wild grasses and some economically important cereals such as triticale, barley, rye, wheat and oat. After the infection, the fungus transforms the developing grain with an alkaloid-containing hard black tuber-like wintering structure called sclerotium. The sclerotia can be harvested together with the cereals and grass and can thus lead to the ergot alkaloid contamination of food and feed products. Ingestion of ergot alkaloids can cause severe health problems. Two main types of diseases are vasospastic gangrenous (e.g. edema, gangrene) and convulsive form (e.g. mania, psychosis, nausea). The current EU regulation sets only maximum levels for ergot sclerotia in certain feed and food. Legislative limits for EAs haven´t been established yet. However EFSA recommends collecting data on the six predominant ergot alkaloids and their epimers in feed and food. Based on the monitoring data collected, the regulatory limits of ergot alkaloids in feed could be established. Thus, there is a need for methods that allow determination of these dangerous toxins. A method based on ultra performance liquid chomatography-tandem mass spectrometry (UPLC–MS/MS) was used for the simultaneous determination of ergometrine, ergotamine, ergosine, ergocornine, ergocristine, ergocryptine and their corresponding epimers. Extraction procedures are based on modified QuEChERS approach (for feed samples) and mixture of water and acidified acetonitrile (for sclerotia). The presented methods were used to inspect various cereals and to determine the EAs content in rye sclerotia. Regarding the cereals results the highest amounts of ergot alkaloids were found in triticale and oat samples. The results of individual rye sclerotia confirm the fact that EAs content in sclerotia is very variable. The total content was observed up to 1.1% which is in compliance with literature data.

[1] Diana Di Mavungu et. al, Food Chemistry 135 (2012) 292–303.
[2] Zachariášová et. al, Analytical Chimica Acta 662 (2010) 51–61
[3] Bolechova et. al.,Food Chemistry 170 (2015) 265–270

Keywords: *ergot alkaloids, feed, Rye Sclerotia*

J37
MYCOTOXIN CONTAMINATION IN ORGANIC FEED MATERIALS IN THE CZECH REPUBLIC

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Since 1989 the Czech organic farming has been continuously growing and in 2014 reached almost 12 % of the farmland acreage. The crops of great importance are cereals followed by fodder crops. Organic products are grown in compliance with the principles of organic farming that typically exclude the use of artificial fertilizers, fungicides and herbicides. This fact could influence fungal growth and subsequent mycotoxin production. Good agricultural practice such as crop rotation, soil preparation and weed control typical for organic farming are critical management tools for minimizing mycotoxin contamination. Since 2010 Central Institute for Supervising and Testing in Agriculture has become the control authority responsible for official controls in organic farming in the Czech Republic. Feed and raw materials for feed production from organic farming are investigated for mycotoxin occurrence. The multiresidue mycotoxin method based on the unbuffered QuEChERS method and ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) represents useful tool for feed analysis. The method has been validated for determination of nivalenol (NIV), deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2), ochratoxin A (OTA), enniatin A (EnA), enniatin A1 (EnA1), enniatin B (EnB), enniatin B1 (EnB1), fumonisin B1 (FB1), fumonisin B2 (FB2), beauvericin (Bea), zearalenon (ZEN), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). This study presents results of both conventional and organic feed samples tested between 2014 and 2015. Frequency of mycotoxin occurrence in organic cereals (wheat, oat, barley) compare to the same convential ones is similar. The most frequent mycotoxins in wheat, oat and barely were enniatin A1, enniatin B, enniatin B1 and beauvericin. In addition T-2 and HT-2 were abundant in oat samples. Maize and maize sillage, grown only in conventional production, have typical profiles with high frequency of presence of Fusarium toxins (zearalenon, fumonisins, T-2 and HT-2 toxins, and beauvericin). These profiles differ from wheat, oat and barley profiles. Medians of concentration levels for inspected mycotoxins in organic cereals are low. They are comparable to the concentration medians of conventional samples except of maize and maize silage. Maize and maize silage (harvest 2014, conventional production) are characterised by high content of zearalenon, deoxynivalenol and beauvericin.

[1] Bolechova, M., Benesova, K., Belakova, S., Caslavsky, J., Pospichalova, Mikulikova, R.: Determination of seventeen mycotoxins in barely and malt in the Czech Republic. Food Control 2015, 108-113

Keywords: *mycotoxins, organic farming, animal feed*

J38
SIMULTANEOUS DETERMINATION OF DEOXYNIVALENOL, ZEARALENONE AND THEIR MODIFIED CONJUGATES IN CEREALS

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Cereal contamination by mycotoxins is a serious problem, and the determination of these substances is an important part of food quality control. Besides the main mycotoxins, the modified mycotoxins have recently come into focus. Particularly deoxynivalenol (DON) and zearalenone (ZEA), both produced by fusarium, were conjugated with glucoside or sulfate within the plant metabolism. 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON) and Nivalenol (NIV) are derivatives of DON; however, they are also part of the investigated spectrum. The toxicity of the conjugates is still being researched. First studies conclude that Deoxynivalenol-3-glucoside (D3G) is less toxic than DON, both in vitro and in vivo [1,2]. However, during digestion it can be hydrolysed back to the mycotoxin DON. Considering this fact, it is important to look for the mycotoxin conjugates, too. A total of 19 beers, 57 malt and 145 maize samples were investigated. The samples were prepared with a modified QuEChERS approach. No manual clean-up was used to avoid substance loss. The analytical method consisted of a two-dimensional LC–MS/MS system with online clean-up. The maize samples were contaminated most frequently. In approximately half of the samples DON, the modified form D3G and ZEA were found. In contrast, only one malt sample was contaminated with DON, D3G and ZEA. The remaining malt and all tested beer samples were free of mycotoxins and their conjugates. In certain cases the lack of available standards complicated the quantification of the modified mycotoxins. As new modified forms are discovered constantly, the availability of standards is necessary.

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[2] De Nijs M, Van den Top HJ, Portier L, Oegema G, Kramer E, Van Egmond HP, Hoogenboom LAP, World Mycotoxin Journal, 2012, 319–324

Keywords: *modified mycotoxins, "masked" mycotoxins, cereals, two-dimensional LC–MS/MS*

J39
TROPANE AND ERGOT ALKALOIDS IN GRAIN-BASED PRODUCTS FOR INFANTS AND YOUNG CHILDREN IN THE NETHERLANDS IN 2011–2014

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Cereals can be contaminated with mycotoxins and plant toxins during growth and harvest. An LC–MS/MS multi-method was developed and in-house validated to simultaneously quantify 20 ergot alkaloids (EAs) and 6 tropane alkaloids (TAs) in cereal-based food products. The method has an limit of detection of 0.1–0.2 µg/kg for most of the EAs and of 0.2–0.5 µg/kg for the TAs. The method was subsequently used to analyse 113 cereal-based food samples (breakfast cereals, biscuits and cookies) for infants and young children. To assess yearly variation, samples were collected in 2011, 2012 and 2014. EAs were detected in 54% of all samples and TAs in 22% of the samples. Mean EA levels in the three sampling years were 10.6, 6.2 and 8.6 µg/kg, respectively, with a maximum level of 115.4 µg/kg. Based on these results and recommended use, exposure to EAs would not have exceeded the health based guidance values set by EFSA in 2012. Mean TA levels were 3.9, 2.4 and 0.4 µg/ kg in the respective years, with a highest level of 80.8 µg/kg. The results indicate that the acute reference dose (ARfD) for TAs, derived by EFSA in 2013, would have been exceeded by young children when consuming some of the products sampled in the years 2010–2012. Over the years the contamination of EAs in cereal-based food products for infants and young children remained at similar levels, but TA levels had decreased drastically in 2014. The latter may be the result of the scientific opinion published by EFSA in 2013, which may have prompted producers to take measures and install adequate quality control of their supplies. The results show the advantage of applying multi-methods in food analysis for detection of potential risks.

Keywords: *tropane alkaloids, ergot alkaloids, cereal based food, young children, LC–MS/MS*

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J40
SIMULTANEOUS ANALYSIS OF MULTI-MYCOTOXIN IN COW URINE AND BLOOD BY LC–MS/MS

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Mycotoxins are toxic substances produced by the metabolism of certain fungi (*Aspergillus, Stachyobotris, Penicillium, Fusarium, Cephalosporium*, etc.). Mycotoxins accumulation in food and feed can be a severe health risk both to humans and animals for their carcinogenic, mutagenic, teratogenic or toxic properties. In addition to mycotoxicosis which is caused by direct consumption of contaminated food and feed, the effect of "carry over" of mycotoxins to animal tissues, milk and eggs should not be disregarded. The measurement of mycotoxin in urine and blood is a useful means of assess individual's exposure than the indirect estimation from average dietary intakes. In this study, a new analytical method was developed and validated for simultaneous analysis of aflatoxin B1 (AFB1), deoxynivalenol (DON), fumonisin B1 (FMB1), ochratoxin A (OTA), and zearalenone (ZEN) in cow urine and blood by liquid chromatography tandem mass spectrometry (LC–MS/MS). Urine and blood samples were purified and concentrated by a double cleanup approach, using a multitoxin immunoaffinity column and a reversed-phase SPE Oasis HLB column. The method gave recoveries in the range 82.5–99.0% when applied to spiked urine and blood samples. The limits of detection were 18.38 ng/g for AFB1, 34.94 ng/g for DON, 11.88 ng/g for FMB1, 4.26 ng/g for OTA, 18.84 ng/g for ZEN in cow urine and 36.15 ng/g for AFB1, 29.31 ng/g for DON, 65.58 ng/g for FMB1, 18.45 ng/g for OTA, 27.74 ng/g for ZEN in cow blood. In conclusions, we used LC–MS/MS method for the analysis of several toxicological important mycotoxins in cow urine and blood. The method could be used in animal pilot studies to monitor simultaneous exposure to the major mycotoxins. However, this study needs to be extended in order to understand the relation between the mycotoxin intake and mycotoxin levels in urine and blood.

Keywords: *mycotoxin, cow, urine, blood, LC–MS/MS*

J41
CHANGES IN THE GENE EXPRESSION OF PYRUVATE DEHYDROGENASE AND α-KETOGLUTARATE IN RAT PRIMARY HEPATOCYTES IN RESPONSE TO MONILIFORMIN EXPOSURE

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The mycotoxin Moniliformin (MON) is produced by nearly 40 different *Fusarium*-species, mainly in maize and grain products in different geographical areas. MON has not been associated with natural outbreaks, however, in vivo acute toxicity in experimental animals have been reported. According to our previous studies, MON is acutely toxic to rats with an LD50 cut-off value of 25 mg/kg b.w. The rats that died showed clinical signs of sudden heart failure (Jonsson et al., 2013). In a subacute oral study, low doses (below 9 mg/kg b.w. MON) did not induce any clinical signs in rats; however, the phagocytic activity of neutrophils was reduced in all tested concentrations (3–15 mg/kg b.w.)(Jonsson et al., 2015). MON is structurally related to pyruvate and is thought to inhibit the incorporation of pyruvate into the TCA cycle and thereby interfere with the energy metabolism of the cells (Pirrung and Nauhaus, 1996). To study the effect of MON to the key enzymes of glycolysis and the TCA cycle on gene level, preliminary gene expression studies were performed using TRAC-technology (PlexPress Oy). The expressions of pyruvate dehydrogenase and α-ketoglutarate in rat primary hepatocytes were down regulated 70% and 30%, respectively, in response to MON. This may indicate that the toxic effect of Mon is not only based on inhibitory effects on enzyme level, but also have direct or indirect implications on gene level. Confirmatory analyses will be performed with RT-qPCR gene expression analysis. The effects of MON will be addressed and discussed further in the poster presentation.

Keywords: *mycotoxins, moniliformin, toxicology, fusarium*

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J42
GROUP DETECTION OF DON AND METABOLITES BY AN ELISA KIT

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Mycotoxins are secondary metabolites produced under field conditions by many species of Fusarium. Among them, Deoxynivalenol (DON), together with the other trichothecenes, is one of the major contaminants of cereals (e.g. corn, wheat, barley) and cereal-based products. DON also occurs in chemically modified forms after plants, animals and fungi metabolization. Up to date natural occurrence of its acetyl-derivatives, 3-acetyl-deoxynivalenol (3Ac-DON), 15-acetyl-deoxynivalenol (15Ac-DON) and glucoside-conjugate deoxynivalenol-3-glucoside (DON-3Glc) has been reported (Berthiller et al. 2013). These metabolites are commonly analysed by chromatographic methods. Indeed, coupled with proper extraction and clean-up, LC-MS represents the best approach for multiresidual characterization of these mycotoxins. On the other hand, immunochemistry-based methods are possibly able to detect a family of structurally related compounds, although the characterization of single contributions is not possible so far. However, ELISA methods often lead to an overestimation of the mycotoxins content, because modified forms and matrix components can potentially cross-react with the antibodies (designed for the parent toxin). Several data about the possible cross-reactivity of commercial DON-detecting ELISA kit are reported in the literature so far. Data are commonly obtained in buffer solutions or in matrix-matched solutions, but comparison on a set of naturally incurred samples has never been reported. In the present work the accuracy of commercial DON-detecting ELISA kit was evaluated on naturally incurred soft wheat (n=15) and maize (n=15), taking into account the matrix effect. Recovery was calculated considering the DON concentration found by LC–MS/MS and the total DON concentration, expressed as the sum of DON and its modified forms found by LC–MS/MS. The obtained data clearly show that, when 3-modified forms of DON occur in the sample, the ELISA kit does actually detect them, thus returning an apparent overestimation if only DON content is considered. When the ELISA recovery is calculated on the total DON content, the accuracy of the analysis increases and the variability decreases. According to our data, the ELISA kit seems to be a promising group detection tool for the accurate evaluation of DON and its modified forms, expressed as sum of DON, DON-3Glc and 3Ac-DON, for soft wheat and maize samples.

[1] Berthiller F, Crews C, Dall'Asta C, De Saeger S, Haesaert G, Karlovsky P, Oswald IP, Seefelder W, Speijers G, Stroka J (2013) Masked mycotoxins: a review. Molecular Nutrition & Food Research 57, 165–186

Keywords: *mycotoxins, enzyme-linked immunosorbent assay, deoxynivalenol, cereals*

J43
MONITORING THE PERFORMANCES OF TECNA'S ELISA TEST KITS FOR MYCOTOXINS THROUGH PROFICIENCY TEST PARTICIPATION

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The last step in the process of development of a new ELISA test kit in Tecna includes validation on matrices. After sample preparation development, in order to set the specificity and sensitivity of the assay, the accuracy, the intra-assay precision and the intermediate variability, a validation scheme is implemented. To perform a proper validation, incurred materials are preferred to spiked ones, since they are considered more representative in routine analysis. Among naturally contaminated materials, the ones characterized within inter-laboratory trials and proficiency tests are preferred, when available. Apart from the in-house validation, all Tecna test kits are submitted to a scheduled monitoring plan over the years. Therefore, performances are kept under control by attending proficiency tests provided by different suppliers (FAPAS, UK; Test Veritas, I; Bipea, Fr; AIA, I). The aim of this poster is to present the results obtained during the past years, by Tecna and with ELISA test line kits I'screen AFLA M1, I'screen OCHRA, Celer® AFLA B1, Celer® DON and Celer® FUMO test kits. 31 aflatoxin M1 contaminated milk and cheese samples were analysed with I'screen AFLA M1 kit. The mean z-score obtained was 0.3, with a minimum value of -0.7 and a maximum of 1.0. 15 materials were analysed for ochratoxin with I'screen OCHRA, 6 were wine and 9 were cereals, obtaining a mean z-score of 0.6 and -0.9 respectively. The minimum z-score for wine was -0.2, the maximum 1.5; for cereals the minimum was -2.0, the maximum 1.53. 14 maize samples were analysed for aflatoxin B1 with Celer® AFLA B1. The mean z-score turned to be 0.2, having a minimum value of -0.7 and a maximum of 0.9. 7 maize materials were analysed for deoxynivalenol with Celer® DON v3 ELISA test kit since 2011. The average z-score was 0.1, with a minimum of -1.4 and a maximum of 1.7. 7 maize samples were analysed for total fumonisins with Celer® FUMO, gaining a mean z-score of 0.6. This is the only kit where a z-score higher than 2 was obtained once (2.1), while minimum z-score was -0.7. Thanks to such monitoring, I'screen and Celer® kits performances are kept under strict control over the years, ensuring the inter-batch quality and the maintenance of the specifications.

Keywords: *proficiency test, quality ensurance, mycotoxins, ELISA kit, z-score*

J44
FIT-FOR-PURPOSE IMMUNOCHEMICAL TEST KITS FOR FUMONISINS SCREENING: DIFFERENT SOLUTIONS FOR DIFFERENT NEEDS

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The aim of the present work is to present the performances of three different immunoassays developed for the screening of fumonisins in maize and other matrices. Celer® FUMO is a quantitative ELISA test kit designed for the analysis of feed and maize by-products intended for animal consumption, meeting the EU and FDA fumonisins limits in these matrices ranging between 1,000 and 60,000 ppb. Celer® FUMO measuring range is 750–60,000 ppb indeed. Since it is addressed to farms, breeders and industries, the assay is fast: it takes 20 minutes. The assay was validated according to Commission Regulation (EU) No 519/2014, by setting the Screening Target Concentration at 1,000 ppb. 20 blank maize samples (< 250 ppb, HPLC analysis) were analysed to set the specificity of the assay in matrix. 100% specificity was obtained, since no false positive results were obtained. The same samples were spiked with 1,000 ppb of fumonisin B1: all determinations were revealed as positive by the assay, with no false compliant results. The bias was evaluated by analysing 150–47,000 ppb contaminated incurred samples, the recovery was 102±23% (n=60). I'screen FUMO is a supersensitive quantitative ELISA test kit conceived for the analysis of maize for human consumption, where EU and FDA limits range between 200 and 4,000 ppb. The assay measuring range is far more sensitive: 25–1,000 ppb, extendable to 5,000 ppb by dilution. Due to its high sensitivity, it was not possible to set any Screening Target Concentration and to analyse the matrix effect of blank maize samples, since no materials with concentration lower than 150 ppb was found. The accuracy of the assay was hence evaluated by analysing 250–5,000 ppb contaminated incurred samples. The mean recovery was 115±24% (n=45). Smart Strip FUMO is a quantitative lateral flow device designed for easy and fast maize acceptance control The main requirement for the development of this assay was quickness, therefore the analysis takes five minutes only. The kit measuring range is 150–4,000 ppb, to be extended to 20,000 ppb by dilution: such measuring range can fit the needs for analysis of raw materials for food and feed production. For the same reason mentioned above, no blank samples were available for the investigation of matrix effect and the sensitivity of the assay. The performances of the kit were investigated by analysing 150–5,000 ppb contaminated incurred samples. The mean recovery was 110±29% (n=78).

Keywords: *fumonisins, maize, elisa kit, lateral flow, validation*

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J45
PRESENCE OF ZEARALENONE IN WHEAT- AND CORN-BASED PRODUCTS PRODUCED IN SERBIA

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Zearalenone is chemically a phenolic resorcyclic acid that is primarily an estrogenic fungal metabolite. The major species of fungus responsible for producing this mycotoxin is Fusarium graminearum. Most often the compound is found in corn, but it is also found in other important crops such as wheat, barley and rice. Generally, the Fusarium species grow in moist, cool conditions and similarly invade crops under these more favorable conditions. The objective of this study was to investigate the presence of Zearalenone in wheat- and corn-based products produced in Serbia. Commercial samples were collected between January 2013 and June 2015 and analyzed by high performance liquid chromatography coupled with fluorescence detection (HPLC-FLD). Samples were prepared by immuno-affinity clean-up column. The method used for detection of the mycotoxins in wheat and corn - based products obtained a recovery rate from 90% to 100%, and limit of quantification was 0,5µg/ kg. During named period, we have analyzed 4491 samples for presence of zearalenone. In 2013, 0.4% of analyzed wheat- and corn-based products were contaminated by zearalenone, while in 2014 it was 6% and in 2015 18%. Concentration ranges were, retrospectively 0.92–6.23 µg/kg for 2013, 0.55–36.5 µg/kg for 2014 and 0.6–47.2 µg/kg for 2015. Maximum level for zearalenone according to Commission Regulation (EC) No 1881/2006 is 50 µg/kg, which hasn´t been exceeded, but we noticed increased presence of zearalenone in 2015. Despite the low contamination observed in wheat- and corn-based products, monitoring the presence of mycotoxins in foods is important to ensure safety.

Keywords: *mycotoxin, zearalenone, wheat- and corn-based products, HPLC–FLD, Immuno-affinity clean-up*

J46
OCCURRENCE OF MYCOTOXINS IN CEREALS CONSUMED IN THE MACARONESIAN ARCHIPELAGOS (CANARY ISLANDS, CAPE VERDE, AND MADEIRA)

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Mycotoxins are toxic substances produced by fungi that are frequently detected in food products, particularly in those whose main ingredient are cereals. Human exposure to mycotoxins is mainly caused by the intake of contaminated food. Therefore quantification of mycotoxins in cereals used as raw material for food preparation is of concern. PERVEMAC is a Cooperative Research and Development Project granted by the European Union, which was built on an unprecedentedly broad and comprehensive program of monitoring pesticide residues and mycotoxins in plant products consumed in the Macaronesian archipelagos. Sampling of cereals was made taking into account the pattern of food consumption in each region, and the number of samples was decided on the basis of the number of inhabitants in each region. Thus, in the Canary Islands 118 cereal samples (wheat flour, oats, bran, spelt, and gofio, a toasted cereal flour that is part of the traditional diet of the archipelago) were taken; in Cape Verde 38 samples (rice, maize, wheat flour, and kamoka, similar to Canary gofio) were taken; and in Madeira 36 samples (maize and wheat flour) were taken. Here we present the results of the occurrence of Aflatoxins B_1, B_2, G_1 and G_2 (AFB1, AFB2, AFG1, and AFG2), Fumonisins B_1 and B_2 (FB1 and FB2) Ochratoxin A (OTA), Deoxynivalenol (DNV) and Zearalenone (ZEA) in these samples. 62% of samples showed at least one mycotoxin, and 33.8% had more than one (2 to 6). Worryingly, 4.2% of the samples exceeded MRLs: AF (3 samples) and FB (5 samples), all of them from Madeira. The type of food most often contaminated was the canary gofio (79%) and the least contaminated was wheat flour (34%). By regions: the highest incidence of mycotoxins was detected in the Canary Islands and the lowest in Cape Verde. The maximum contamination level of total AF was present in a sample of wheat flour from Madeira (12.4 µg/kg), which also exhibited the highest level of contamination by total FB (2445 µg/kg). FB was type of mycotoxin exhibiting the highest concentrations of all, and it also was the most frequently detected. On the contrary, OTA was the least frequently detected, as it was only present in a sample (canary gofio, 0.3 µg/kg). As regards DNV, gofio was also the type of food that showed the highest concentration (92.6 µg/kg). This mycotoxin was not detected in any of the samples from Madeira, while in samples from the Canary Islands and Cape Verde it was detected with similar frequency (24.7% and 31.4%, respectively). Finally, a similar situation was observed for ZEA, which was not detected in any of the samples from Madeira, and occurred similarly in samples from the Canaries and Cape Verde (highest concentration = 22.1 µg/kg in a sample of corn from Cape Verde). Our results revealed a high percentage of positive samples and indicate that continuous monitoring by multiple laboratories is effective and necessary to minimize human intake of these harmful toxins.

Keywords: *mycotoxins, gofio, potential exposure levels, intake assessment, tolerable daily intakes (TDI)*

Acknowledgement: *European Regional Development Fund (ERDF). European Territorial Cooperation programs: PCT-MAC*

J47
ASSESSMENT OF MYCOTOXIN INTAKE THROUGH THE CONSUMPTION OF THE TOASTED CEREAL FLOUR CALLED "GOFIO", A TRADITIONAL FOOD OF THE CANARY ISLANDS, SPAIN

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"Gofio" is a type of flour made from toasted grain, usually corn or wheat, which is part of the staple food in the Canary Islands, Spain. It has been demonstrated that this food has many nutritional properties, and even that is cardioprotective. However, a recent study reported that the gofio was the cereal-based food with the highest concentrations of mycotoxins (fumonisins B1 and B2) among more than 1200 samples from France, Germany, and Spain. Since this food can be potentially consumed regularly by more than 2 million inhabitants of the Canary Islands, and also occasionally by more than 10 million tourists visiting the archipelago each year, the quantification of mycotoxins in gofio is of concern. In the context of PERVEMAC Project, which is a Cooperative Research and Development Project focused on the impact of the presence of PPP residues and mycotoxins in plant products consumed in the geographical area of Macaronesia, 69 gofio samples were obtain ed in supermarkets in the Canary archipelago, and the occurrence of Aflatoxins B1, B2, G1 and G2 (AFB1, AFB2, AFG1, AFG2), Fumonisins B1 and B2 (FB1 and FB2) Ochratoxin A (OTA), Deoxynivalenol (DNV) and Zearalenone (ZEA) was evaluated. Data obtained were used to estimate the potential exposure levels using the upper bound approach for average and high consumers (95th percentile), both for adults and children, and these estimates were compared with the tolerable daily intakes (TDI). 78.3% of the samples were contaminated with at least one mycotoxin and 68.9% of the analyzed samples showed co-occurrence of mycotoxins (2 to 6 residues per sample). Although the high incidence of mycotoxins in these samples, the concentrations were well below the established MRLs in all the cases (maximum values of total AF=0.14 µg/kg; total FB=178.3 µg/kg; OTA=0.3 µg/kg; DNV=92.9 µg/kg; and ZEA=9.9 µg/kg). For adults the daily dietary exposure to total AF was estimated to be 0.06 ng/kg bw for average consumers and 0.17 ng/kg bw for high consumers, which amounted 6.1% and 16.7 % of the TDI, respectively. For total FB the estimated intakes for average and high consumers were 27.2 ng/kg bw (1.4% TDI) and 74.1 ng/kg bw (3.7% TDI), respectively. For the rest of the mycotoxins the estimates for both types of adult consumers were: OTA=0.01 ng/kg bw (0.06% TDI), and 0.03 ng/kg bw (0.17% TDI); DNV=23.5 ng/kg bw (2.3% TDI), and 63.7 ng/kg bw (6.4% TDI); and ZEA=2.5 ng/kg bw (1% TDI), and 6.6 ng/kg bw (6.6% TDI). For children all estimates almost double those of adults. This means it could be the case that a child who is a major consumer of this food could be exposed to almost 35% of TDI of aflatoxins only through the consumption of gofio. Therefore, the results of this study indicate that it would be necessary that health authorities remain vigilant and articulate appropriate measures to reduce the presence of mycotoxins in this important food for the Canary Islands population.

Keywords: *mycotoxins, gofio, potential exposure levels, intake assessment, tolerable daily intakes (TDI)*

Acknowledgement: *European Regional Development Fund (ERDF). European Territorial Cooperation programs: PCT-MAC*

J48
OCCURRENCE OF FUSARIUM MYCOTOXINS IN HYBRID TRITICALE LINES

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Mycotoxins are naturally occurring secondary metabolites of fungi colonizing kernels of cereals. They have been studied intensively due to their occurrence in food and feed, and from here their potential threat human and animal health. The appearance and accumulation of mycotoxins can vary from year to year depending on many factors such a weather conditions agricultural practices and varieties. In Poland Triticale is the second most important cereal crop and a major component of animal feeds. Poland has great achievements in breeding new varieties. They are currently working on breeding hybrid varieties. The hypothesis assumes that hybrids are different from open pollinated varieties susceptible to the accumulation of mycotoxins in the grain. The aim of the study was to compare the amount of mycotoxins in the grain line of hybrid and open pollinated winter triticale. The experimental materials consisted of two winter triticale cultivars Palermo and Wiarus and three lines of triticale hybrid DAST 22/11 F1, CM 12/10 F1, SM 4/11. The analyzed grain samples, were obtained from field experiment conducted at the Experimental Station in Osiny (51°35'N, 21°55'E) belonging to Institute of Soil Science and Plant Cultivation - National Research Institute in Puławy in the growing season of 2013/2014. Triticale grains were naturally infected by fungal pathogens producing mycotoxins. Detection and quantification of deoxynivalenol (DON), T-2/H-T2 toxin and zearalenone (ZEA) in triticale was carried out using Enzyme Linked Immunoassay (ELISA). It was found that the grains of triticale contained all three of the investigated mycotoxins (DON, ZEA and T-2/H-T2). In comparison of two types of triticale the least resistance to mycotoxins were lines of triticale hybrid. Contamination of DON was very hight and was in grain of Palermo - 1100µg/kg, Wiarus -1250 µg/kg, DAST 22/11– 2975µg/kg, CM 12/10 – 3300 µg/kg,SM 4/11–1950 µg/kg. In all of analyzed samples the safe level of contamination of DON which is 1250 µg/kg, was exceeded: 2,4 times for DAST 22/11 F1, 2,6 times for CM 12/10 F1 and for SM 4/11 was 1,6 times. In the case of T-2/H-T2 and ZEA, contamination of grain samples was comparable. The largest amount of ZEA was observed in samples of SM 4/11 and was 34,4 µg/kg but the lowest level of that mycotoxin in Wiarus and was 4,7 µg/kg while the m ost abundant in toxin T-2/H-T2 proved to be CM 12/10 F1. Our studies show that triticale hybrid is highly susceptible to presence of mycotoxins for that it can be harmful for animals as a feed.

Keywords: *mycotoxins, triticale hybrid*

J49
DIFFERENT STRATEGIES OF SAMPLE PREPARATION IN MULTI-MYCOTOXIN ANALYSIS IN ANIMAL FEED

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The multi-mycotoxin analysis in feed samples are challenging due to heterogeneity of the samples and number of matrix constituents interfering with analytes signals. Thus, effective sample preparation is still crucial part of analysis. The aim of the study was comparison of different sample preparation techniques used in the multimycotoxin analysis in feed ("dilute and shoot" approach,"QuEChERS – based" clean-up, solid phase extraction and immunoaffinity clean-up with cartridges designed for multi-mycotoxin analysis). The 5 g of ground feed (spiked with nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, HT-2 toxin, T-2 toxin, ochratoxin A, fumonisin B1 and B2, aflatoxin B1, B2, G1 and G2, sterigmatocystin and zearalenone) was extracted with 20 ml of acetonitrile:water:acetic acid (79:20:1) solution in 30 min. Next, sample was centrifuged and the supernatant was clean-up with different schemes. In the "dilute and shot" approach, 0.5 ml of extract was diluted with 5 ml of mobile phase solution. In the "QuEChERS-based" clean-up, 1 ml of extract was added to the Eppendorf tube contained different combination of salts (MgSO4 and NaCl) and sorbents (C18, active carbon, PSA) and vortex-mixed. In the SPE scheme (reverse-phase), 2 ml of extract was mixed with 9 ml of water and passed through conditioned C18, Hybrid SPE and Oasis cartridges. The analytes were eluted with 3 ml of methanol. In the SPE scheme (normal-phase), 5 ml of extract passed through conditione d amino, and SiOH cartrigdes. The analytes were eluted with 3 ml of methanol: water (7:3). In the immunoaffinity clean-up, 1 ml of extract was diluted with 9 ml of PBS buffer and passed through conditioned cartridges: AOF-MS-PREP and DZT MS-PREP (R-Biopharm©) connected in tandem. The analytes were eluted with 1 ml of methanol and 1 ml of water. The extracts were evaporated (N2, 40°C), mixed with labelled internal standards solution and determinated with UHPLC–MS/MS technique. The results show that all above scheme are suitable for multi-mycotoxin determination in animal feed, but none of them is perfect solution. For the "dilute and shot" and "QuEChERS-based" clean-up, the main problem was the low signal to noise for HT-2 and aflatoxins. Such challenges were not observed for immunoaffinity clean-up, but important drawback of this technique is limited number of covered analytes (e.g. lack of signal for nivalenol) and and high cost of cartridges. SPE-approach is effective for most analytes but shows low recoveries for polar mycotoxin like deoxynivalenol and nivalenol (reversed-phase SPE) and hydrophobic like sterigmatocystin (normal-phase SPE). In conclusion, for effective multi-mycotoxin determination in animal feed, at least two different schemes of sample preparation should be combined e.g. "QuEChERS-based" clean-up with selective immunoaffinity columns.

Keywords: *mycotoxins, LC–MS/MS, feed, sample preparation*

J50
RAPID IDENTIFICATION OF POISONOUS ENTOLOMA RHODOPOLIUM AND EDIBLE E. SARCOPUM MUSHROOMS USING PCR-RESTRICTION FRAGMENT LENGTH POLYMORPHISM AND REAL-TIME PCR

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The family *Entolomataceae Kolt*. & *Pouzar*, which belongs to the order Agaricales, has three main genera: *Rhodocybe Maire, Clitopillus (Fr. ex Rabenh) P. Kumm*. and *Entoloma (Fr.) P. Kumm. s. l.,* and is composed of more than 1,500 species and distributed worldwide. Mushrooms of this family vary in size, shape, and color, but have pink gills and pinkish or pinkish-brown spores in common. *Entoloma rhodopolium (Fr.) P. Kumm, Entoloma salmoneum (Peck) Sacc*. and *Entoloma strictius (Peck) Sacc.,* were poisonous. *Entoloma sarcopum Nagasawa & Hongo* (synonym *Rhodophyllus crassipes* (Imaz et Toki) Imaz et Hongo) is one of edible mushrooms, especially in Japan. However, E. *sarcopum* resembles closely the poisonous *E. rhodopolium* and E. *sinuatum*. Therefore, poisoning occurs many times every year in Japan and other countries. To add to the difficulty of morphological identification between species, E. rhodopolium is reported to have six forms in western North America, and we found at least four morphologically different forms in Japan. Classification of the *Entoloma* mushrooms needs to be reconsidered. To reduce human poisoning by these mushrooms, easy and rapid identification methods are strongly required. The taxonomy of *E. rhodopolium* and its related species are complicated due to their diversity. Detailed phylogenic study has been required for the Entoloma mushrooms. Therefore, we performed classification study. We first collected *E. rhodopolium*, *E. sinuatum* and *E. sarcopum* species from various regions of Japan, from Hokkaido (northern) through Shimane (southwestern) to cover genetic variation among these mushrooms in the country. We show that mushrooms morphologically identified as *E. rhodopolium* or *E. sinuatum*, by experts fell into four classifications, three clades of E. rhodopolium and *E. sarcopum* by a molecular phylogenic study. Based upon the results of phylogenic analysis, we next developed a rapid identification method using PCR-Restr iction Fragment Length Polymorphism (RFLP) to clearly determine the all species including edible E. sarcopum, by a pattern of electrophoresis analysis using restriction endonucleases MslI, DdeI, and HincII-HaeIII. MslI digestion reveals discrimination of edible and poisonous mushrooms, whereas DdeI, and HincII-HaeIII provide species identification in the poisonous E. rhodopolium clades. The rapid and simple PCR–RFLP method can provide reliable identification immediately after collection from fields. It takes only a few hours, and is expected to decrease poisoning. To identify species from mushrooms in cooked and/or ingested food samples, which contains fragmented DNA, we also developed an additional PCR–RFLP against a shorter target sequence with MslI site. The method provides determination of edible and poisonous mushrooms very rapidly. Moreover, we have developed a real-time PCR method for identification of these four Entoloma species. Results will be discussed in detail.

Keywords: *mushroom, entoloma, PCR–RFLP, Real-time PCR*

J51
DETERMINATION OF 30 PYRROLIZIDINE ALKALOIDS AND RELATED N-OXIDES IN PLANT MATERIALS USING LC-Q-ORBITRAP-MS ANALYSIS

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Pyrrolizidine alkaloids (PAs) are a group of food contaminants which are exclusively biosynthesized by plants. So far more than 600 different PAs and PA N-oxides are known and it has been estimated that approximately 6000 plant species, representing 3% of all flowering plants, are able to express PAs. Typical plant families are Boraginaceae, Asteraceae and Fabaceae. PAs are derived from the chemical structure of pyrrolizidine and can be sub-grouped into retronecine-, heliotridine-, crotanecine-, supinidine- and otonecine-type PAs. In particular, the 1,2-unsaturated compounds are known to expose hepatotoxic effects and they are under suspicion to be genotoxic and cancerogen. Common sources for PA intoxications of humans and livestock are phytopharmaceuticals, food and feed supplements, herbal teas, milk, honey and silage. In these commodities high concentrations of up to several mg/kg can be found. So far, no regulatory limits have been defined for this group of contaminants in food and feed. In the past, numerous different analytical approaches have been applied for the analysis of PAs. Next to a sum parameter method which is based on a Zn / LiAlH4 reduction followed by GC–MS analysis of the necine base backbone, common liquid-solid extraction methods followed by cleanup and target-analysis using LC–MS/MS have been described. [1,2] As an alternative to these often very time consuming approaches a fast dilute-and-shoot method followed by LC–Q-Orbitrap analysis for PA identification and quantification in plant materials was developed and will be presented. The sample preparation was carried out by a liquid-solid extraction of homogenized sample materials using a mixture of acidified water/methanol followed by shaking, centrifugation and dilution of the supernatant. For analysis, an Ultimate 3000 UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer was used. The mass spectrometer was operated in fullscan + data dependen t MS2 acquisition mode (ESI+) with a resolving power of 70,000 FWHM. The analytes were identified by verification of their exact mass, isotopic pattern and MS/MS fragmentation. As there are no internal standards available on the commercial market so far, quantifications were performed using matrix matched calibrations. In order to assess the fitness-for-purpose of the method a validation was performed by PA fortification of blank herbal tea extracts in a concentration range of 0.5–10 ng/mL (representing 10–200 ppb sample concentration). The linearity was found to be very good with $r^2 \geq 0.9994$ for 29 out of 30 compounds. The precision and LOQs of the method was determined by a six fold determination of fortified extracts at 10 ppb and 40 ppb. The results show an RSD of 1.4±0.7 % and LOQs of 2.6±1.2 ppb. Further performance parameters regarding the analytical method will be presented on the poster.

[1] EFSA 2011; EFSA Journal, 9(11):2406.
[2] Bodi et al. 2 014; Food Addit. Contam. Part A, 31(11):1886–1895

Keywords: *pyrrolizidine alkaloids, LC–Q–Orbitrap-MS, food contaminants, plant material, herbal tea, plant toxins*

J52
ENNIATINS: EMERGING TOXIC METABOLITES OF FUSARIUM MICROMYCETES

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Agricultural production is often negatively influenced by the occurrence of microscopic filamentous fungi and their toxic secondary metabolites, mycotoxins. Cereals belong to the most frequently affected agricultural commodities, and in the moderate climatic zone, Fusarium fungi/mycotoxins dominate. While for several mycotoxins of this group, the maximum levels have been legislatively established (namely deoxynivalenol (DON), zearalenone (ZON), and fumonisins B1 and B2 (FB1, FB2)), regulations for enniatins, mycotoxins emerging during the recent years due to the climatic changes, are still lacking. Since 2010, they have become the subject of interest of the European Food Safety Authority (EFSA) who has organized several calls for data about their occurrence in food and feed.

The present study involves altogether 665 cereal samples (507 and 158 wheat and rye samples, respectively) collected between the years 2011 and 2014, in which 57 mycotoxins, including the emergent enniatins, were analyzed. For the isolation of target analytes, the optimized QuEChERS-based extraction, followed by ultra-high performance liquid chromatography coupled with tandem mass spectrometric separation and detection (Veprikova et al., J. Agric. Food Chem., 2015) was enabled. DON, previously considered as the marker of mycotoxin contamination of cereals, was detected in approx. 50% of the wheat samples and 40% of the rye samples only. On the other hand, occurrence of enniatins in the samples was significantly more frequent (i.e.78% of wheat samples, and 95% of rye samples positive for enniatin B). The maximum concentration level of the sum of enniatins reached up to thousands of µg/kg. The observed extent of enniatins contamination indicates the need to pay more attention to this scientific research topic.

Keywords: *mycotoxins, enniatins, cereals, QuEChERS, U-HPLC–MS/MS*

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J53
DEVELOPMENT OF A LC–MS/MS METHOD USING ATMOSPHERIC PRESSURE PHOTOIONIZATION TO DETERMINE VARIOUS PHENYLPROPANOIDS, (FURO-)COUMARINS, PULEGONE AND MENTHOFURAN IN HERBAL-BASED PRODUCTS

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Herbal-based products have gained increasing relevance in livestock since the European ban (2006) of antibiotics, other than coccidiostats or histomonostats, used as growth promoting agents in animal nutrition. As an alternative to improve productivity in livestock, herbs and herbal-based products have been investigated in in vitro and in vivo studies. However, minor attention is drawn to the occurrence of undesirable substances in such feedingstuffs. A literature review revealed two major groups of secondary plant metabolites present in herbal plants of possible concern. Firstly, phenylpropanoids like estragole, methyleugenol and safrole with genotoxic and carcinogenic effects. Secondly, phototoxic furocoumarins, e.g. bergapten, xanthotoxin, and hepatotoxic coumarins. These substances are widely spread across herbal plant families. Furthermore, two monoterpenes, pulegone and its metabolite menthofuran, were identified because of their hepatotoxicity and their presence in some herbal plants. A novel LC–MS/MS method was developed in order to simultaneously determine various phenylpropanoids, furocoumarins, coumarins pulegone and menthofuran in herbal-based products. During development and optimization of the instrumental method, dopant assisted atmospheric pressure photoionization showed the best results to cover all analytes in one analytical method. LC–MS/MS parameters were optimized, as well as different sample preparations techniques for herbal matrices, including extraction and clean-up procedures. After validation, the method is intended to analyse herbal-based products for animal feed currently available on the market.

Keywords: *method development, atmospheric pressure photoionization, LC–MS/MS, secondary plant metabolites, herbs*

J54
5 MINUTES QUANTITATIVE DETECTION OF FUMONISINS IN MAIZE AND DDGS BY SMART STRIP FUMO

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Smart Strip FUMO is a new lateral flow device developed for the quantitative detection of fumonisins in cereals and by-products. The assay was developed in order to provide farmers and feed mills with a reliable test strip together with a suitable cost-effective reader. Smart Strip FUMO was hence developed as a 5 minutes assay, with a measuring range of 150 – 4,000 ppb that can be extended to 20,000 ppb by diluting the sample extract. The assay cross-reactivity was established for the three main fumonisins, turning to be 100% for fumonisin B1, 70% for fumonisin B2 and 106% for fumonisin B3. The applicability of the assay was verified for maize and maize DDGS. Briefly, maize samples were ground and homogenized prior extraction. 10 g of sample was extracted with 30 ml of 70% methanol by shaking for 3 minutes. The sample was then filtered on Whatman 1 filter paper and diluted 1:3 in proper dilution buffer. Since DDGS samples have much lower pH than raw cereals, an adjustment was necessary upon extraction using 1M NaOH. 100 µl of extract was run on the strip (5 minutes at room temperature), then the reaction result was read by means of LAB LFD READER, a multiplex scanner connected to a PC that is able to acquire up to 5 assays in parallel at the same time. Three blank maize samples (<100 ppb total fumonisins, HPLC analysis) were analysed, three replicates each. No matrix effect was found. 20 incurred maize samples (250–4,000 ppb total fumonsins, HPLC analysis) were analysed to study the accuracy of the assay. The mean recovery was 110±29%. Since three replicates were run for each test material, the mean variability was found to be 18%. The accuracy of the developed assay was also tested with maize DDGS samples. Samples were spiked at three different levels and analyzed in triplicate on two different days. The overall recoveries were 98−101% and the coefficients of variations were lower than 21%. Ten naturally contaminated DDGS samples (1,400–6,000 ppb total fumonisins, UHPLC–MS/MS analysis) were also analysed in triplicate on two different days.). The correlation of LFD and UHPLC–MS/MS results was very good and the mean recovery for all the analyzed samples was 115±14%.

Keywords: *fumonisins, maize, DDGS, lateral flow, validation*

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J55
PYRROLIZIDINE AND TROPANE ALKALOIDS IN TEAS AND THE HERBAL TEAS PEPPERMINT, ROOIBOS AND CHAMOMILE IN THE ISRAELI MARKET

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Dehydro Pyrrolizidine alkaloids (dehydro PAs) are carcinogenic phytotoxins prevalent in the Borginaceae, Asteraceae and Fabaceae families. Dehydro PAs enter the food and feeds chain by co-harvesting of crops intended for human and animal consumption as well as by carry-over into animal-based products such as milk, eggs and honey. Recently the occurrence of dehydro PAs in teas and herbal teas has gained attention from the European Union, due to the high dehydro PAs-levels found in commercially available teas and herbal teas in Germany and Switzerland. Furthermore, several tropane alkaloids (TAs, e.g. scopolamine and hyoscyamine) -intoxications due to consumption of contaminated herbal teas were reported in the literature. The aim of the present study was to determine the dehydro PAs and TAs-levels in 70 pre-packed tea-bags of herbal and non-herbal tea-types sold in supermarkets in Israel. Chamomile, peppermint and rooibos teas contained high dehydro PAs levels in almost all samples analyzed. Lower amounts were detected in black and green teas, while no dehydro PAs were found in fennel and melissa herbal teas. Total dehydro PAs concentrations in chamomile, peppermint and rooibos teas ranged from 20 to 1729 µg/kg. Except for black tea containing only mono-ester retrorsine-type dehydro PAs, all other teas and herbal teas revealed mixed patterns of dehydro PA ester types indicating a contamination by various weed species during harvesting and/or production. The TA levels per tea bag were below the recommended acute reference dose, however the positive findings of TAs in all peppermint tea samples warrant a more extensive survey. The partially high levels of dehydro PAs found in teas and herbal teas present an urgent warning letter to the regulatory authorities to perform routine quality control analysis and implement maximum residual levels for dehydro PAs.

Keywords: *pyrrolizidine alkaloids, tropane alkaloids, LC–MS/MS, tea, herbal tea*

J56
IDENTIFICATION OF ´NEW´ MASKED MYCOTOXINS: OCCURRENCE OF GLYCOSILATED ALTERNARIA TOXINS IN TOMATO BASED PRODUCTS

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Many foreign substances, including mycotoxins, are metabolized in the living organisms in order to reduce their toxicity. During the recent years, conjugation of deoxynivalenol, zearalenone (*Berthiller et al., International Journal of Food Microbiology, 2007*) or HT2/T2 toxins (*Veprikova et al., World Mycotoxins Journal, 2012*) with glucose or other polar substances in plants have been proved. In spite of reduced toxicity for plants, those conjugates still pose a risk for mammals, because of the probable cleavage of the parent mycotoxin in the gastrointestinal tract (*Chiara Dal´Asta et al., Chemical Research in Toxicology, 2013*), which underlines the relevance of the 'masked' mycotoxins research. Within our study, we focused on the development of method for analysis of conjugated ('masked') *Alternaria* mycotoxins in tomatoes and tomato products. The method comprised the aqueous extraction, separation on the reversed-phase liquid chromatography (Accucore aQ 100 x 2.1 mm; 2.6 μm particle size; Thermo Scientific) and detection by ultra-high resolution mass spectrometer (Q-orbitrap, Thermo Scientific). Identification of *Alternaria* toxins conjugates was based on the measurement of the exact mass and elemental composition calculation. As the confirmatory criteria, the agreement of isotopic patterns, and fragment spectra in high resolution, were taken. The *Alternaria* toxin glucosides and sulphates were screened in a wide set of tomato-based samples available from the Czech market (35 samples of tomato paste, 11 samples of tomato juice, and 5 samples of dried tomatoes). As a result, we were able to identify the alternariolmethylether-monoglucoside (for the first time in naturally contaminated samples) in the majority of the samples investigated.
In the second part of the study, also the free *Alternaria* toxins were determined by using the QuEChERS-based isolation (*Lacina et al., Journal of Chromatography A, 2012*) and U-HPLC–HRMS/MS separation/detection. In the samples, alternariol (AOH), alternariolmethylether (AME), tentoxin (TEN) and tenuazonic acid (TEA) were determined with a frequency of occurrence 63, 43, 33 and 14%, respectively. The concentration ranges in positive samples were 0.7–89.7μg kg^{-1} for AOH, 0.7–51.5 μg kg^{-1} for AME, 56.3–1957.7 μg kg^{-1} for TEA, and 2.3–5.3 μg kg^{-1} for TEN.

Keywords: *alternaria mycotoxins, masked mycotoxins, tomato based products, U-HPLC–HRMS/MS*

Acknowledgement: *Financial support from specific university research (MSMT No 20/2015)*

J57
CHALLENGES AND LIMITATIONS OF TANDEM HIGH RESOLUTION MASS SPECTROMETRY IN ANALYSIS OF PLANT ALKALOIDS IN FOOD MATRICES

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Tropane and pyrrolizidine alkaloids belong to the group of naturally food contaminants. These toxic secondary metabolites occur in plants of Brassicaceae, Solanaceae and Senecio family. In addition to (-)-hyoscyamine and (-)-scopolamine, the main tropane alkaloids, over 200 tropane alkaloids are known. Similarly, besides of heliotrine, motocrotaline, lycopsamine, retrosine, senecionine, seneciphylline, senkirkine or their N-oxides, the most well-known representatives of pyrrolizidine alkaloids, approximately 600 different species have been until now identified. The recent EFSA effort to assess health risk associated with dietary intake of these compounds has encountered the problem due to a lack of relevant occurrence data. Relevant information about their occurrence has not been completed yet, partially because of the lack of proper analytical methods. That is why in our study, we focused on development of the multi-detection approach for determination of various tropane / pyrrolizidine alkaloids within one analytical run.
As regards extraction/purification step, several ways of sample treatment were tested (e.g. the simple aqueous-methanol extraction or the QuEChERS-based procedure), with the aim to minimize the number of involved operations and assuring good recoveries of a wide range of targeted compounds. For an effective analytes separation, several types of ultra-high performance liquid chromatographic columns, employing different modifications of C-18 based stationary phases, and different particle types were examined. As concerns detection strategy, our main focus was on a comparison of two conceivable mass spectrometric techniques, modern high resolution tandem mass spectrometry, represented by quadrupol-orbitrap mass analyzer (Q ExactiveTM Plus, Q-OrbitrapTM , Thermo SCIENTIFIC), and the 'gold' mass spectrometric standard, unit resolution tandem mass spectrometry represented by triple quadrupol mass analyzer (QqQ, Triple Quad 6495, Agilent). Both the advantages and drawbacks of each particular detection approach, including the detection sensitivity at trace levels, possibilities of results confirmation, performing of the non-target screening and retrospective data mining, were critically assessed. The method enabling simultaneous analysis 35 target analytes (24 tropane and 11 pyrrolizidine alkaloids) have been developed and optimized for several matrices, including cereals, pseoudocereals, or 'difficult' matrices such as herbal tea mixtures. The performance characteristics as recoveries, limits of detection, repeatabilities, and matrix effects will be discussed in relation to the sample preparation, as well as detection approaches.

Keywords: *tropane alkaloids, pyrrolizidine alkaloids, ultra-high performance liquid chromatography, high resolution mass spectrometry, tandem mass spectrometry*

Acknowledgement: *Financial support of the project funded by EFSA, GP/EFSA/BIOCONTAM/2014/01 – GA 1: 'Occurence of tropane alcaloids in food' is gratefully acknowledged.*

J58
IMPLEMENTATION OF SAMPLE PRETREATMENT PROTOCOLS FOR THE QUANTITATIVE ANALYSIS OF MARINE BIOTOXINS PRESENT IN COMPLEX SEAFOOD SAMPLES

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Marine biotoxins are natural contaminants of the marine environment mostly affecting bivalve molluscs. The complexity of the matrices in which these toxins are present is the main limitation when looking for an efficient analytical protocol, being necessary to take into account the recovery correction in order to ensure the reliability of the analytical results. The efficiency of the extraction is clearly compromised by the interferences present in the matrix, being the interferences removal a critical step that need to be evaluated. The transition from qualitative to quantitative methods clearly demands the implementation of analytical methods, emphasizing the need for protocols including steps that allow to remove interferences that could compromise the interpretation of the analytical result, on the other hand the inclusion of these steps, makes strictly necessary to take into account results corrected for recovery. This work has been focused in two group s of marine toxins for which chemical methods are being used as reference methods in the EU, Amnesic shellfish poisoning (ASP) and Lipophilic toxins which have to be analysed by Liquid Chromatography coupled to UV detection (LC–UV) and to tandem Mass Spectrometry (LC–MS/MS) respectively. The results obtained for the evaluation of different sample pretreatment strategies, mostly based on solid phase extraction (SPE, dispersive SPE, etc) are presented in this work and from these results it can be concluded that there is a need for improved analytical protocols, in particular for analyzing processed bivalves which increased complexity, and results corrected for recovery are strictly required to ensure the reliability of the analysis carried out.

Keywords: *Solid phase extraction, marine toxins, processed bivalves*

NANOPARTICLES

(K1 – K7)

K1
DESIGNING THE ROLLING CIRCLE AMPLIFICATION BASED SURFACE-ENHANCED RAMAN SPECTROSCOPY METHOD FOR 35S PROMOTER MAIZE GENE DETECTION

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In this study, we combined two novel methods: rolling circle amplification (RCA) and surface-enhanced Raman spectroscopy (SERS) to detect genetically modified organisms on maize sample. Firstly, rolling circle amplification part of study was obtimised. Then, gold surfaces and gold nanorods were used to form sandwich structure. SERS enhancement were provided using 5,5'-Dithiobis(2- Nitrobenzoic acid) (DTNB) label. The target concentrations were quantified via SERS spectra of DTNB on the nanorods. RCA reaction amplified the obtained SERS signal and the detection limit was found to be 6.3 fM. The specificity tests were performed using 35S promoter of Bt-176 maize gene and nonsense sequence. Due to the obtained results, the developed RCA-SERS based sandwich assay method is quite sensitive, selective and specific for target sequences in model and real systems.

Keywords: *rolling circle amplification (RCA), surface enhanced Raman spectroscopy (SERS), 5,5'-Dithiobis(2- Nitrobenzoic acid) (DTNB), gold nanorods*

Acknowledgement: *The Scientific and Technological Research Council of Turkey; Project number: 111T096*

K2
EVALUATION OF THE PRESENCE OF NANOMATERIALS IN A VARIETY OF FOODS AND DRINKS BY DLS, AF4-MALLS–ICP–MS AND SP–ICP–MS

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Nanoparticles (NPs) can be present in consumer food products as anticaking agent or as white pigment for example. Regulations of the European Union currently establish that the word "nano" should be included in the label of the product if NPs are present at 50 % or more in the size range from 1 nm to 100 nm. For the moment, for presence of calcium carbonate (E170) and vegetable carbon (E153) NPs is allowed, but in the following years (2015-2018), other additives that could be added in a nano-form will be evaluated, such as titanium dioxide (E171), iron oxides and hydroxides (E172), silver (E174), gold (E175), silicon dioxide (E551), calcium silicate (E552), magnesium silicate (E553a) and talc (E553b). The aim of the work is to develop a methodology for the evaluation of the presence of NPs in different foods and drinks and compare the results obtained by different techniques: Dynamic Light Scattering (DLS), Asymmetrical Flow Field-Flow Fractionation coupled with Multiangle Laser Light Scattering and Inductively-coupled Plasma Mass Spectrometry (AF4-MALLS–ICP–MS) and Single Particle Inductively-coupled Plasma Mass Spectrometry (SP–ICP–MS). Firstly, analysis of NPs reference materials of diverse nature, metallic and non metallic (polystyrene nanospheres, Au, SiO_2 and TiO_2) has been studied for method validation. Then, foods and drinks were analyzed after sample preparation. Different protocols have been applied depending on the sample: i) extraction with water (sugar coated chocolate candies, chewing gum), ii) suspension in water (cappuccino powder), iii) filtration (energetic drinks, wines, beer, fruit juices, coffee, hot chocolate). According to DLS, the size of the partic les obtained in the drinks ranged from 10 to 300 nm that could correspond to polysaccharides, micelles, biopolymers or metallic NPs. Metal contained in the NPs can be identified by SP—ICP–MS or AF4-MALLS–ICP—MS. TiO_2 was found in chocolate candies and chewing gum and both techniques can be used and give a similar size between 80–200 nm. SiO_2 particles are present in cappuccino powder, coffee and hot chocolate. However, SiO_2 particle size determination is troublesome by SP–ICP–MS due to interference problems causing a high limit of detection (LOD: 200 nm). Satisfactory results were obtained with AF4-MALLS–ICP–MS for reference material of colloidal silica (ERM FD-102), but Si concentration seems too low in the selected samples to obtain information about the size.

Keywords: *nanoparticles, drinks and foods, AF4-MALLS, SP–ICP–MS, DLS*

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K3
ASSESSING NANOPARTICLE MIGRATION FROM COMMERCIAL FOOD CONTACT MATERIALS INTO AQUEOUS FOOD SIMULANTS

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An emerging category of nanotechnology enabled consumer products is that of food-related products, which include nanomaterials in food as well as food contact materials (FCMs), Assessing the risks associated with a food contact product involves the evaluation of the overall health effects of raw nanomaterials, and the potential for nanoparticle release into food over time, due to diffusion or expected material wear during normal use. While most research has focused on studying the potential health effects of various nanomaterials, there is a dearth of data on release rates and potential consumer exposure. The few studies in the literature evaluating nano-containing FCMs have reported conflicting results regarding nanoparticle release. A number of researchers do not detect nanomaterials migrating into the food simulant; an equal number do report nanoparticle migration. This study was developed to evaluate the differences in these observed outcomes and understand the role of food simulants in these seemingly contradictory results. The stability of silver nanoparticles spiked into three food simulants (water, 10% ethanol and 3% acetic acid) was investigated using orthogonal analytical techniques. Asymmetric field flow fractionation (AF4), ultrafiltration, electron microscopy (EM), and single particle inductively coupled mass spectrometry (sp-ICP-MS) data showed significant oxidative dissolution of silver nanoparticle in 3% acetic acid which was not observed in water and 10% ethanol. This suggests that 3% acetic acid may potentially impact observed outcomes when used as a simulant for evaluating potential silver nanoparticle migration from FCMs.

Keywords: *migration, silver nanoparticles, food simulant, stability, sp-ICP-MS*

Acknowledgement: *AF4*

K4
DETECT, CHARACTERIZE AND QUANTIFY ENGINEERED NANOMATERIALS IN FOOD AND FOOD PACKAGING BY SINGLE PARTICLE ICP–MS

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Nanotechnology is making its way to food and food packaging. The use of nanomaterials in food is a highly contentious topic as many people are diametrically opposed to the concept of consuming foodstuffs "tainted" by nanotechnology. Metal and metalloid particles are used for a variety of purposes in the food chain, silicon dioxide (coffee creamer and anticaking agent), Titanium dioxide (tooth paste and various candies) and copper nanoparticles (antibacterial properties that aid in the prevention of several food-borne diseases and keep food fresh). This work describes the theory and application of Single Particle-ICP–MS in analyzing Metal-Based Nanoparticles in various food items, TiO_2 in tooth paste and Ag in dietary supplement products as an example. Single Particle ICP–MS (SP–ICP–MS) differentiate ionic and particulate signals, quantitate both fractions, measure particle concentration (part/mL), composition, particle sizes, size distribution and track dissolution and agglomeration, all through a single interface using the Syngistix™ Nano Application Module.

Keywords: *nanomaterials, single particle ICP–MS, metal based nanoparticles*

K5
ISOTHERMAL AMPLIFICATION OF PLASMONIC GOLD NANOPARTICLES FOR THE DETECTION OF *CAMPYLOBACTER* AND *SALMONELLA* SPECIES

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Campylobacter and *Salmonella* are the most common foodborne pathogens which cause a serious threat to food safety. Potential implications of foodborne pathogens are severe human illness and economic loss. The challenges connected with bacterial infections include the complex ecology, biology, genetic variability and low infective dose with high pathogenicity. These factors make pathogenic bacteria difficult to control, as well as being extremely dangerous to human health. It is evident that detection methods must be highly sensitive and specific to assist detecting the low quantity of bacterial cells found in food.
Classical methods for the detection and identification of bacteria relies on sample pre-enrichment, and subsequent culturing of the sample on selective agar media. This approach is time-consuming (4–7 days), labor-intensive, less sensitive, and requires special laboratory facilities and trained staff. Molecular diagnostic methods using PCR, DNA microarrays, and DNA sequencing have greatly improved the speed, sensitivity and specificity of bacteria detection. However, these methods are still cumbersome, expensive and also time-consuming. In both the classical and molecular approaches, samples must be transported to a near-by diagnostic laboratory, increasing the time of obtaining a final result. This time, along with the time taken to complete the analysis leaves an entire community or a large part of a population exposed to potentially contaminated foods. Therefore, to comply with food security and prevention of Campylobacteriosis and Salmonellosis, a rapid, sensitive and specific diagnostic test for the detection of bacteria, at or near the site of the poultry meat production is highly required.
Herein, we present an innovative approach based on isothermal amplification of plasmonic gold nanoparticles (AuNPs) for the detection of pathogenic bacteria. The current work utilises the localised surface plasmon resonance (LSPR) properties of AuNPs which are functionalised with specific RNA capture probes for *Campylobacter* or *Salmonella*. In the presence of target DNA, the RNA-AuNP hybridises forming a heteroduplex which becomes a template for RNAse H cleavage of the RNA probe. This liberates the target DNA allowing it to re-hybridise with another RNA probe on the nanoparticle surface. The target recycles under isothermal conditions until all of the RNA is cleaved, resulting in bare nanoparticles. Upon addition of NaCl, the bare nanoparticles are aggregated, initiating a colour change from red to blue, due to a decrease in interparticle distance.
The contribution of pathogenic bacteria to globally important foodborne illnesses has major implications for the safety of the food supply chain. The current method is expected to provide appropriate and timely measures to help identify and remove infected animals or foods from the production chain, thus mitigating outbreaks and economic losses, and improving the protection of human health.

Keywords: *pathogens, nanoparticles, nanotechnology, biological sensor*

K6
TESTING SILVER MIGRATION FROM COMMERCIALLY AVAILABLE NANO-SILVER FOOD CONTAINER INTO FRESH CHICKEN MEAT UNDER PLAUSIBLE DOMESTIC STORAGE CONDITIONS

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The development of nanotechnology derived food packaging materials to improve food preservation is one of the most interesting and promising application of nanotechnology in the agri-food sector. Among different nanomaterials (NMs) that are currently exploited in order to prolong food shelf-life, Silver nanoparticles (AgNPs) have acquired relevant importance, since recent studies have proven that Silver antibacterial activity is kept or even increased in the nanoparticles (NPs) form due to the enhanced reactivity resulting from the high surface/volume ratio, which is typical of NPs. However, the direct contact of food with packaging incorporating NPs may result in the migration of such materials into the food. This represents a matter of concern since only limited data are available about the safety of NMs and their potential impact on consumers' health. So far, several migration studies have been carried out on different polymeric food packaging materials containing AgNPs, proving that different parameters may affect migration into food simulants such as temperature, containers characteristics and simulants composition. Equivalent studies on real food matrices, simulating real life scenarios, are currently missing. Aim of this study was to evaluate Ag migration from a commercially available food packaging containing AgNPs into a real food matrix under plausible domestic storage conditions. For this purpose chicken meatballs wrapped in nanoSilver (presence and morphology were verified by scanning electron microscopy) and control plastic bags were stored up to seven days at 5 and 12°C and for 35 days at -18°C before being thawed both at 22 and 5°C. Due to the fact that characterization and quantification of NPs in complex matrices is still a really challenging issue, two analytical methods based on the determination of total Ag were developed and validated in meat matrix, by means of Atomic Absorption Spectroscopy and Inductively Coupled Plasma Mass Spectrometry respectively. Validation was performed on fortified blank meat at 20, 50, 75, 100 ng/g using a known AgNPs dispersion following the conventional validation approach required for quantitative confirmatory analysis in agreement with Commission Decision 2002/657/EC. The methods, characterized by good trueness, precision, ruggedness and sensitivity, enable to detect silver concentrations down to 5 ng/g, were therefore considered fit for purpose. Chemical analysis revealed the absence of Ag in meatballs under the experimental conditions, in contrast with previous studies on stimulants where higher experimental temperatures and matrices characterized by higher polarity were used, confirming that different factors may play a key role in Ag and AgNPs migration. This study allowed to highlight the importance of performing migration tests also simulating real life usage condition to assess the actual consumer exposure scenario.

Keywords: *nanothecnology, food packaging, Silver, migration*

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K7
CHARACTERIZATION OF SILICON DIOXIDE AND TITANIUM DIOXIDE NANOPARTICLE INTERACTIONS WITH WHEY AND WHEY PROTEINS

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Silicon dioxide, an anticaking agent and titanium dioxide, a whitener, are nanomaterials that may be potentially used in the food sector. Their interaction with the food matrix and the fate in the gastrointestinal tract is largely unknown. Silicon dioxide as well as titanium dioxide nanoparticles, similarly to other nanoparticles, are expected to interact with compounds of their environment resulting in the adsorption of such compounds onto the nanoparticle surface. The resulting "corona" may affect the properties and behavior of the nanoparticles in a food matrix. Therefore a detailed analysis of the interaction between nanoparticles and the food matrix is of utmost importance. The binding of single whey proteins (BSA and β-lactoglobulin) to silicon dioxide and titanium dioxide nanoparticles was analyzed. The effect of the chemical structure and surface characteristics of the nanoparticles on the interaction with whey and whey proteins was investigated. It was shown that titanium dioxide nanoparticles interact with unprocessed whey stronger than silicon dioxide nanoparticles. Titanium dioxide nanoparticles bound lactoferrin with highest affinity among all the proteins whey consists of. Binding of single proteins was confirmed with several methods. An increase in the hydrodynamic diameter and changes in zeta potential were registered after addition of BSA and β-lactoglobulin to aqueous suspensions of the nanoparticles. Interactions of nanoparticles with proteins were shown to be dependent on the protein species and on the chemical properties of nanoparticles. Further investigations are needed in order to characterize the nature of these specific interactions.

Keywords: *protein corona, titanium dioxide nanoparticles, silicon nanoparticles, whey*

NOVEL FOODS AND SUPPLEMENTS

(M1 – M12)

M1
CONTINUOUS PRODUCTION OF PREBIOTIC PECTIC OLIGOSACCHARIDES FROM SUGAR BEET PULP IN A CONTINUOUS CROSS FLOW MEMBRANE BIOREACTOR

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Pectic oligosaccharides (a class of prebiotics) are non-digestible carbohydrates which benefits the host by stimulating the growth of healthy gut microflora. Production of prebiotic pectic oligosaccharides (POS) from pectin rich agricultural residues involves a cutting of long chain polymer of pectin to oligomers of pectin while avoiding the formation of monosaccharides. The objective of the present study is to develop a two-step continuous biocatalytic membrane reactor (MER) for the continuous production of POS (from sugar beet pulp) in which conversion is combined with separation. Optimization of the ratio of POS/monosaccharides, stability and productivities of the process was done by testing various residence times (RT) in the reactor vessel with diluted (10 RT, 20 RT, and 30 RT) and undiluted (30 RT, 40 RT and 60 RT) substrate. The results show that the most stable processes (steady state) were 20 RT and 30 RT for diluted substrate and 40 RT and 60 RT fo r undiluted substrate. The highest volumetric and specific productivities of 20 g/L/h and 11 g/gE/h; 17g/l/h and 9 g/gE/h were respectively obtained with 20 RT (diluted substrate) and 40 RT (undiluted substrate). Under these conditions, the permeates of the reactor test with 20 RT (diluted substrate) consisted of 80% POS fractions while that of 40 RT (undiluted substrate) resulted in 70% POS fractions. A two-step continuous biocatalytic MER for the continuous POS production looks very promising for the continuous production of tailor made POS. Although both the processes i.e 20 RT (diluted substrate) and 40 RT (undiluted substrate) gave the best results, but for an Industrial application it is preferable to use an undiluted substrate.

Keywords: *pectic oligosaccharides, residence time, membrane reactor, specific productivity, volumetric productivity*

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M2
NUTRITIONAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SELECTED WILD EDIBLE PLANTS

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Nutritional values and medicinal potential of wild plant foods are of considerable importance, as these help to pinpoint traditional food resources of poor population in developing countries such as Pakistan. However, it is important to scientifically standardize such plants and create community awareness to accept wild food plants as useful as the cultivated ones. This study was performed to determine the nutritional composition, antioxidant and antimicrobial activities of wild plants available in Pakistan. Eight wild plant species namely, Chenopodium murale, Eruca sativa, Goldbachia laevigata, Malcolmia africana, Malva neglecta, Medicago polymorpha, Melilotus officinalis and Nasturtium officinale were collected from their natural habitat and were analyzed. Proximate analysis of plants showed the presence of a good amount of fibres and proteins in M. neglecta and M. officinalis while of the twelve minerals analyzed in which calcium and potassium were predom inant in all plants. The total antioxidant and free radical scavenging activities of the plant species showed a linear co-relation with the total phenolics. Our results indicated that methanolic extracts of plant species exhibited measurable inhibitory effect against Escherichia coli, Salmonella typhi, Streptococcus pneumonia and Proteus vulgaris. These results may be useful for the evaluation of the wild plants for their nutritional values and phytomedicinal potentials. The data will also enable the selected wild plants for their possible applications in functional foods, nutraceuticals and pharmaceutical developments. Moreover, the current work will provide new reference data and will give awareness to the public consuming these unconventional wild plants as food.

Keywords: *wild edible plants, nutritional evaluation, biological potential, Pakistan*

M3
UPGRADING PROTEIN PRODUCTS USING BIOPROCESSING ON AGRICULTURAL CROPS

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Due to increasing world population, higher average income, and changes in food preferences, there is a growing demand for proteins, especially novel plant-based protein sources, that can substitute animal proteins and supplement currently used soya proteins. Increased customer awareness to sustainability leads to a demand for plant protein products made from locally grown crops. Novel bioprocessing methods have been developed to generate protein products which are nutritious, readily available and do not generate hazardous waste. The processing focus has therefore been on developing protein-enriched products with minimized content of antinutritional compounds. For every crop it is a challenge to obtain protein fractions with sufficient added value to make processing economically feasible. In this work we present the characterization of protein products developed in pilot scale using the novel, environmentally-friendly, water-based biofractionation method. The process was optimized to yield products with protein content comparable to existing commercial products. Raw materials used for processing included soya white flakes, wind-sifted pea, lupine seeds and rapeseed pressed cake. For each of these raw materials the process needed to be optimized separately, as they present different challenges for the biofractionation. For example, soya contains proteinase inhibitors, which, if not removed, can decrease digestibility of protein. Likewise, glucosinolates present in rapeseed should be removed from the protein product to ensure good quality. During the processing these unwanted compounds can be separated from the protein to upgrade the quality of the final products. Protein products developed in the pilot scale were analyzed with respect to protein content, protein profile, protein solubility, glucosinolate content (rapeseed product), saponin content (soybean- and pea-derived products) and trypsin proteinase inhibitors (soya and pea product). Using our biofractionation methods allowed the development of protein products from different raw materials and upgrading the protein content in comparison to the raw material.

Keywords: *bioprocessing, plant proteins, food and feed quality, legumes, cruciferous crops*

Acknowledgement: *NyProFood, GUDP-raps, Hitfood, Lehnsgaard, Agrokorn*

M4
AN ELISA TEST FOR THE QUANTIFICATION OF BOVINE LACTOFERRIN IN MILK AND MILK PRODUCTS

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Bovine lactoferrin (bLF) plays a key role in the defence mechanisms of the mammary gland of lactating animals. bLF can inhibit bacteria by chelating iron under certain conditions. Next to this antimicrobial activity, bLF is reported to be a natural antioxidant with numerous other functions, including anti-viral, anti-fungal, anti-inflammatory and anti-cancer activities. Since November 2012, the European Food Safety Authority (EFSA) approved bLF produced by Friesland Campina as “novel food” meaning that it is safe to be used as an ingredient in a variety of foods, including infant nutrition. A competitive enzyme immunoassay is developed for a fast quantification of bLF in milk and milk products. The test is validated for a variety of dairy matrices, such a milk, milk powder and baby milk powder. The limit of quantification (CCβ) in baby milk powder is 10 µg/ml.

Keywords: *lactoferrin, novel food, ELISA, baby milk powder*

M5
OPTIMIZATION OF LIQUID-CHROMATOGRAPHY QUADRUPOLE/TIME-OF-FLIGHT MASS SPECTROMETRY (LC–QTOF) FOR TARGET SCREENING OF ANABOLIC-ANDROGENIC STEROIDS

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Anabolic-androgenic steroids (AAS) are increasingly abused in sports worldwide regardless of their prohibition and possible health complications or hazards. In our study, liquid chromatography coupled to quadrupole/time-of-flight high resolution mass spectrometry (LC–QTOF) has been applied for target screening of AAS particularly their bounded forms like testosterone undecanoate etc. Due to the possibility of full-scan product-ion spectra with high mass accuracy acquisition, QTOF is very valuable tool for identification of unknown compounds in samples. In addition, the evaluation of data based on comparison of retention time, mass accuracy, isotope abundance and isotope spacing results in strong level of identification confidence. In presented study a Personal Compound Database (PCDL) containing CID mass spectra of more than 25 AAS measured at three different collision energies was created. Under optimized conditions, fragmentation ion spectra containing both precursor and two or more product ions were obtained for all of the analytes. The chromatographic separation of the targeted AAS was carried out by liquid chromatography using Kinetex C18 column (100 × 2.1 mm, i.d., 2.6 µm) with gradient mobile phase (water/acetonitrile with 0.1% formic acid). The presented method will be subsequently applied for screening of AAS in adulterated prescription drugs and food supplements.

Keywords: *anabolic-androgenic steroids, LC–QTOF, high resolution*

M6
FUNCTIONAL-TECHNOLOGICAL INDICES OF COMBINED MEAT MINCED HALF-FINISHED GOODS FROM THE FERMENT RAW MATERIAL

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Working on new species of combined food stuffs consumers' preferences are taken as a principle according to a row of indices, characterized a finished product. Generally organoleptic indices and a food value are the most important for consumers. Though working on new species of a production it is also important for a producer to take into account some other indices, which are not important for a consumer, but play a significant part for getting super-profits by a producer. It is possible to refer to such indices functional-technological indices, and in the first place an output of the finished commodity. Taking into account modern tendencies to keep a healthy life-style by people including a desire for the healthy food, a tendency of development healthy food stuffs was widespread. It is eliminated to use in the composition of healthy food stuffs chemical additives. Biotechnology methods make it possible to improve the properties of the meat raw material without using chemical substances by means of the fermentation of the meat raw material. In the research the results of an identification of functional-technological indices of combined meat half-finished products are given. The combined meat half-finished products were made from low-grade meat raw material – the 2nd class beef, which was exposed a preliminary ferment processing. Vegetable oil and oatmeal are introduced to the formulation of half-finished goods in order to increase a food value and give to the product dietary properties. All above listed components are introduced when making minced meat of half finished goods as a preliminary prepared protein-fat emulsion. The results of an organoleptic assessment show that when half-finished goods of fermented meat raw material, oatmeal and vegetable oil were introduced into the minced meat as a protein-fat emulsion, it is ensured an increasing of a damp-binding capacity on 20% on the average, a water-holding capacity on 12%, a fat-holding capacity on 18% in the comparison with the control. At the same time an output of the finished commodity increases on 22% on the average. The results show that an introducing into minced raw material of a protein-fat emulsion, made from a fermented meat raw material, oatmeal and vegetable oil, leads to the improvement functional-technological indices and increasing the output of the finished commodity.

Keywords: *functional-technological indices, fermented raw-material, half-finished goods*

M7
DEVELOPMENT OF THE ANALYTICAL METHOD OF MARKER COMPOUND IN FERMENTED SOYBEAN EXTRACT

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Fermented soybean extract is obtained by hot water extraction of soybeans (Glycine max) fermented with Aspergillus oryzae. This extract has α -glucosidase inhibitory activity that can help to maintain blood sugar levels by inhibiting the absorption of sugar. α-Glucosidase inhibitory activity is attributed to Tris(hydroxymethyl) aminomethane in fermented soybean extract. We selected two methods for analysis of marker compound in fermented soybean extract. One of the methods is enzyme assay for α-glucosidase inhibitory activity. α-Glucosidase inhibitory activity was determined by spectrophotometer at 405 nm using p-Nitrophenyl α-D-glucopyranoside as a substrate. The method measured the half maximal inhibitory concentration (IC50) for inhibition of α-glucosidase in fermented soybean extract ranged from 0.015 to 0.75 mg/mL. The other method is development of the identification method of Tris(hydroxymethyl)aminomethane in fermented soybean extract by HPLC–FLD after derivatization and GC–FID after silylation by N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% Trimethylchlorosilane (TMCS).

Keywords: *fermented soybean extract, α-glucosidase inhibitory activity, tris(hydroxymethyl)aminomethane*

Acknowledgement: *This research was supported by a grant (15161MFDS014) from Ministry of Food and Drug Safety in 2015.*

M8
A NEW ANALYTICAL METHODOLOGY FOR UNKNOWN GENETICALLY MODIFIED ORGANISMS USING LINEAR−AMPLIFIED MEDIATED PCR (LAM–PCR)

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A variety of genetically modified organisms (GMO) have been developed these days. Unlike conventional GMO, endogenous promoters, such as maize ubiquitin, rice actin, and granule-bound potato starch synthase promoters are used in recently developed GMO. In addition, an inserted gene of interest is originated from cross-compatible related species. An increasing number of GM crops are cultivated in developing countries as well as developed countries. Sometimes unintentional contamination of unapproved GM crops occurs, and the contaminated foods may be exported. Many countries have zero tolerance policy for unapproved GMO in food use; therefore they monitor crops and processed foods to be imported at border. A screening test is an effective method for a wide variety of GMO. However, the above-mentioned types of GMO are difficult to detect and identify as GMO. After screening tests, a further analysis like inverse PCR and genome walking methods are forced to perf orm to clarify the presence of GMO whether consciously or not, because the results of screening tests for endogenous promoters cannot conclude contamination. However, inverse PCR and genome walking methods are laborious, meaning that we have to proceed through a trial and error process. To establish sure methodology, we investigated linear-amplified mediated PCR (LAM–PCR) method for this purpose. We targeted a left border sequence (LB) and an endogenous promoter pGBSS. Using GM potato samples containing endogenous promoters and inserted genes, we linearly amplified the sample by a 5'-biotin-labeled primer, and bound it with streptavidin-fused magnetic beads, and then formed double-stranded DNA using random hexanucleotide primer and Klenow polymerase. After endonuclease digestion (MseI or HpyCH4IV), a linker cassette was bound to the digested DNA fragment. Release of unlabeled single strand DNA by NaOH followed by washing with water was done. The resulting single strand DNA was subjected to two-time nested PCR. Final PCR product was analyzed by electrophoresis and sequencing. When we targeted LB sequence, LAM-PCR provided a single band on an agarose gel, and its sequencing analysis revealed the sequence of the junction region between LB and potato genome. When targeting pGBSS, LAM–PCR gave a single band with the same size in non-GM and GM potato. However, sequencing analysis unveiled the difference; GM potato has the sequence flanking pGBSS and part of the inserted gene aspartic acid synthase, the other has not. LAM–PCR method is considered very useful for unknown sequences from short known nucleotides (ca.50 bp) in GMO. This suggests that even multi-copied genetically engineered sequences can be analyzed by the method. Next generation sequencing technology may boost the application then.

Keywords: *LAM–PCR, genetically modified food, endogenous promoter, screening test, GMO*

M9
THE EUROPEAN DECATHLON PROJECT – AN OVERVIEW

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The European Decathlon project (http://www.decathlon-project.eu/) is an ambitious research project, aiming to improve DNA-based methods in three areas, i.e. in the area of the detection of food pathogens, the detection of (unauthorised) genetically modified organisms (GMOs), and with relation to different customs issues. The customs issues relate to both improved methods for the characterisation of tobacco batches, including reconstituted tobacco, as well as to the detection and identification of endangered species under CITES (Convention on International Trade in Endangered Species). One of the ways to realise the aims of the Decathlon project is to achieve synergy among these quite different areas given the universal applicability and robustness of DNA-based detection methods, allowing for similar approaches to be followed. In this concise overview of the Decathlon project the main aims and goals of the project will be presented. The project includes ready-to-use DNA-based methods that fill the gaps in the current tool box, advanced and innovative applications responding to complex analytical questions, as well as pragmatic, on-site applications for use within companies as well as by inspection services. The methods range from isothermal DNA methods for on-site applications to (dd)PCR, LAMP, and a range of advanced NGS (Next Generation Sequencing) approaches coupled to user-friendly bioinformatics pipelines. In addition to this related Decision Support System modules will be developed to guide the user in how to assess analytical results with respect to specific questions, for instance the potential presence of unauthorised GMOs in specific supply chains.

Keywords: *DNA-based method, LAMP, Next Generation Sequencing (NGS), ddPCR, bioinformatics*

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M10
NGS AND DIGITAL PCR AS NEW APPROACHES IN GMO DETECTION

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The current approach of simplex real-time PCR has its limitations for GMO (genetically modified organism) detection. One is the low level of multiplexing. This is an increasing bottleneck for cost-effective GMO detection given the increasing number of GMOs in agriculture. The second is the inability to quantify GMOs reliably at a level of 0.1%, which is required in some cases according to current European legislation. A third one is the inability to detect unknown targets, which is of increasing importance for the detection and identification of unauthorised GMOs (UGMOs). ddPCR results will be shown for a duplex system, endogenous and GM target, demonstrating similar characteristics, such as LOD, as real-time PCR, but without the need of a standard curve. A further increase in cost-effectiveness will be shown by presenting data on the development of optimised multiplex ddPCR methods for quantification of all maize EU-authorised events in one reaction. Also data based on 3D-PCR will be presented. The most cost-effective way of employing NGS in GMO detection at the moment is by identification of enriched targets. Sequencing of a selection of known fragments is expected to improve cost-effectiveness of screening methods. The latest results in this approach will be shown and compared to the cost and time in current screening approaches across the EU. The MySeq NGS system was selected as the most promising table-top platform for re-sequencing short amplicons, and the latest results will be shown. The largest progress in cost-effectiveness will be made by multiplex selection for unknown sequences and NGS. Combining a multiplex genewalking strategy, covering all relevant known GM elements, with the NGS expertise as compiled within the Decathlon project will allow the development of an informative GMO multimethod to detect and identify almost any GMO present in a single sample, including the possible detection of UGMOs. Theoretical and practical data will be presented leading to the most adequate method for (multiplex) genewalking. Since sequencing length and absence of assembly artefacts outweigh the need for >95% accurate base-calling, largely the latest PacBio NGS data will be shown for this topic. Whole genome or transcriptome (re)-sequencing would currently be the only option for identification of GMOs that do not contain any previously known GMO element (Tengs et al., 2009). This approach works when the wild-type genome is sufficiently known and annotated. By in silico subtraction of sequence data that match the non-GM reference genome, such an approach would yield a subset of the sequence data that can be subjected to de novo assembly and successive further analysis to detect and characterize completely unknown inserts and insertion loci. The many potential genetic differences between sample and reference sequences will not only be related to genetic modifications but also to natural genetic variation. Data will be shown on the development of an initial module for a data analysis pipeline based on a theoretical approach on available GMO genomic sequence datasets as well as simulated data based on available genomic data combined with known GMO sequence information.

Keywords: *digital PCR, NGS, cost-efficiency, unauthorised, GMO*

M11
THE MOLECULAR ANALYTICAL AND BIOINFORMATICS TOOLBOX OF DECATHLON

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Nowadays, there are many upcoming DNA-based methods that each has distinct advantages over the current standard approach of real-time PCR. Digital droplet PCR (ddPCR), 3D digital PCR, New Generation Sequencing (NGS), isothermal amplification are alternative and/or supplemental methods which can further advance DNA-based methodologies with relation to many analytical questions. In the Decathlon project advanced molecular analytical and bioinformatics knowledge is compiled from the three areas of the Decathlon project (food pathogens, traceability of GMOs and custom issues). Basic knowledge is exchanged between the three areas of application, for instance related to fundamental characteristics of upcoming methodologies, such as ddPCR, 3D digital PCR and isothermal amplification strategies. In addition to these basic characteristics of available and upcoming NGS platforms are discussed and assessed in terms of applicability, available software solutions and cost-effectiveness with respect to the broad range of identified issues within the Decatlon project all requiring innovative and dedicated strategies and related NGS data analysis pipelines. This section will present recent technical achievements applying different innovative methodologies, targeted strategies and, where applicable, dedicated NGS data analysis modules to answer identified stakeholders' issues in the three areas of the Decathlon project.

Keywords: *isothermal amplification, ddPCR, NGS, bioinformatics, cost efficiency*

Acknowledgement: *This work is funded by Grant No. FP7-KBBE-2013-7-613908-Decathlon under the Seventh Framework Program*

M12
RAPID SCREENING AND CONFIRMATION OF PDE5 INHIBITORS IN DIETARY INGREDIENTS BY DART–MS AMBIENT IONIZATION

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INTRODUCTION Phosphodiesterase type 5 inhibitors (PDE5 Inhibitors) are used to treat erectile dysfunction (ED). Numerous different PDE5's have been detected in herbal supplements, vitamin tablets and adulterated pharmaceuticals. In response to this adulteration problem the AOAC has issued a call for methods focused on development of technologies for rapid determine the presence of various PDE5s in dietary ingredients. This work describes the method we have developed to address this adulteration problem
METHODS Analysis of PDE-5 inhibitor standards at multiple concentrations was completed using a Direct Analysis in Real Time (DART–SVP) ambient ionization source coupled to both a HRMS and a QQQ LC/MS system. Samples and standards were prepared for analysis by dissolving a quantity with solvents. For preparation of adulterated materials, an aliquot of each standard solution was applied directly to the supplement material and allowed to dry before extraction. 33 replicates of each supplement were analyzed. The [M+H]+ with accurate mass assignment was used to confirm PDE5s presence in the screening method. Statistical analysis of the accuracy of the method was assessed for each of the analytes in each type of sample. An MS/MS method was used for the confirmation method.
PRELIMINARY DATA. The possibility of ionization by DART was determined for a collection of PDE5s with confirmation by comparing fragmentation patterns to a database. All of the PDE-5 inhibitors standards were detected at concentrations ten times lower than required. The optimum temperature of the PDE5s was determined with a DART temperature profile. PDE5s were then spiked into dietary supplements at different concentration, extracted into a liquid and analyzed by DART. PDE5s were detected down to the lower limit of 10ppm.
NOVEL ASPECT A method for rapid screening and confirmation of PDE-5 inhibitors in supplement by DART-MS met goals defined by the AOAC

Keywords: *supplements, phosphodiesterase type 5 inhibitor*

PACKAGING CONTAMINANTS

(N1 – N15)

N1
SIMULTANEOUS ANALYSIS OF BISPHENOL A AND OTHER BISPHENOL ANALOGUES IN FOODS BY GC–MS

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Bisphenol A (BPA) is an industrial chemical, and its major applications in food packaging include the production of polycarbonate containers and epoxy resin can coating. BPA has been of concern for human exposure due to its estrogenic activity. Due to consumers' concerns regarding the safety of BPA, other bisphenols may also have been used in food packaging as replacement of BPA, and can be found in foods as well. In this study, a sensitive and selective GC-MS method was developed and validated for simultaneous analysis of BPA and three other bisphenols, bisphenol B (BPB), bisphenol E (BPE), and bisphenol F (BPF). This method was used in a recent survey to analyse samples of 52 canned fish products as a follow up of previous study conducted five year ago to investigate any changes in BPA levels since then and levels of other bisphenols due to possible changes in can coating formulations. BPB and BPE were not detected in any of the 52 canned fish products, while BPF was detected in only four products at low levels from 1.8 to 5.7 ng/g, indicating that BPA is likely still the dominant bisphenol used in current can coating formulation. BPA was detected in all 52 canned fish products, but at much lower levels compared to previous study, ranging from 0.96 to 265 ng/g with an average of 28 ng/g, likely due to changes in can coating formulation from the industries. The few products with high BPA levels (> 100 ng/g) are exclusively from a new brand which is available only recently. Further analysis of canned foods is necessary in the future to capture any changes in BPA levels and the occurrence of other bisphenol analogues.

Keywords: *bisphenol A, bisphenol B, bisphenol E, bisphenol F, canned food*

N2
MIGRATION OF PHOTOINITIATORS FROM CARDBOARD INTO DRY FOOD: EVALUATION OF TENAX® AS A FOOD SIMULANT

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Food packagings, providing physical protection and extending the shelf life of foods, are becoming increasingly complex in their design and composition. The direct contact, and in many cases the indirect contact, between the food packaging and the food, yield the transfer of relative low molecular weight compounds such as photoinitiators. Photoinitiators are widely used to cure ink on packaging materials used in food applications such as cardboards for the packaging of dry foods, but residues of unreacted photoinitiators are able to migrate from the printed food contact material in the food. Evaluation of the migration of chemical contaminants in food is necessary but very challenging due to the complexity of the matrix and the wide variety of foods that need to be analysed. If a food contact material is not yet in contact with food, the contact material can be checked for compliance using food simulants. According to the Regulation (EU) No 10/2011, the official simulant for dry food is poly(2,6-diphenylphenylene oxide), also known under its commercial name Tenax®. Conventional migration testing for long term storage at ambient temperature with Tenax® was applied to paperboard for the following photoinitiators: benzophenone (BP), 4,4'-bis(diethylamino)benzophenone (DEAB), 2-chloro-9H-thioxanthen-9-one (CTX), 1-chloro-4-propoxy-9H-thioxanthen-9-one (CPTX), 4-(dimethylamino)benzophenone (DMBP), 2-ethylanthraquinone (EA), 2-ethylhexyl-4-dimethylaminobenzoate (EDB), Ethyl-4-dimethylaminobenzoate (EDMAB), 4-hydroxybenzophenone (4-HBP), 2-hydroxy-4-methoxybenzophenone (HMBP), 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (HMMP), 2-isopropyl-9H-thioxanthen-9-one (ITX), 4-methylbenzophenone (MBP) and Michler's ketone (MK). Test conditions (10 days at 60°C) were according to Regulation (EU) No 10/2011 and showed different migration patterns for the different photoinitiators, which are probably caused by the presence or absence of certain functional groups. The results were compared with the migration in cereals after a storage of 6 months at room temperature. The simulation with Tenax® at 60°C overestimated actual migration in cereals up to a maximum of 92%. Nevertheless, replacing the Tenax® food simulant by real food (rice) does not result in accurate simulations because underestimations for the actual migration were made for BP, CTX, EDMAB and MBP, showing Tenax® is a much stronger adsorbent than foods, concluding its suitability as a food simulant.

Keywords: *Tenax®, kinetics, cereals, photoinitiators*

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N3
DEVELOPMENT OF A COMPREHENSIVE AND HIGHLY SENSITIVE GC–MS/MS MULTI-METHOD FOR THE ANALYSIS OF PLASTICISERS IN FATTY FOODS

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Plasticisers are widely used as additives in plastic polymers to obtain required technological properties. If food comes into contact with such plastics, either during processing or through their use as packaging materials, the plasticisers can migrate into the food and contaminate it. Due to the hydrophobic nature of plasticisers fatty foods, such as edible oils, pestos, etc., are especially prone to such contamination. Most laboratories analysing for plasticisers focus on only one group of them, the phthalates. Although these are widely used and some of them pose health concerns, several other chemical groups exist which are utilised as plasticisers and at least partially replace the phthalates. These compounds include adipates, sebacates and citrates. Therefore, it seems advisable to target not only phthalates in plasticiser analysis but to employ a broad screening approach encompassing different plasticiser groups. Moreover, EU regulation 10/2011 contains a provision whereby several plasticisers from different chemical groups must not exceed a summed specific migration limit. Thus, we have developed a GC–MS/MS based multi-method with which more than forty plasticisers from different chemical classes (phthalates, adipates, sebacates, citrates and others; including several isomeric mixtures) can be determined in fatty foods. The method provides not only a broad scope but also high sensitivity with limits of quantification reaching 10 µg/kg for most analytes. The various steps of method development will be outlined and the various challenges encountered will be discussed. Special focus will be placed on the optimisation of the MS/MS transitions which was crucial for obtaining the required selectivity and sensitivity in the complex oil matrix. The fact that a high-resolution chromatographic separation is of great importance despite the selectivity offered by tandem mass spectrometry will also be highlighted. Finally, the results of method validation will be presented, giving details on the method's characteristics.

Keywords: *plasticisers, multi-method, GC–MS/MS, edible oils*

N4
DETERMINATION OF THE MIGRATION OF SULPHONATED RESIDUES FROM POLYSULPHONE BABY BOTTLES USED AS SUBSTITUTES FOR POLYCARBONATE

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From 2012, the European Commission and the U.S. Food and Drug Administration have both banned the sale of baby bottles that contain bisphenol A (BPA), an oestrogen mimicking chemical that could adversely affect brain and reproductive development in foetuses, infants and children. As a consequence, plastic substitutes have appeared in the Southeast Asian market. Polysulphone baby bottles in particular were highly favourable, as it was thought to be more resistant to leaching. Unfortunately, Bisphenol S (BPS), which is the main starting material in polysulphone baby bottles, has now routinely been identified to be present in human blood test. Recent studies published in the Environmental Health Perspectives have also demonstrated BPS ability to mimic oestrogen in female lab rats, just like BPA. In other words, BPS can cause similar endocrine disruption at the same low level as BPA. Considering their structural similarities, there is a strong case to evaluate t he safety of the potential replacement for polycarbonate with polysulphone baby bottles. This work presents an analytical method for identification and quantification of four chemicals found in polyethersulphone (PES) and polyphenylsulphone (PPSU) baby bottles. The method was applied to the migration testing of 30 baby bottles found in the Southeast Asian market made of PES and PPSU. The potential starting materials of interest in baby bottles are diphenyl sulphone (DPS), 4,4'-dichlorodiphenyl sulphone (DCPS1), 2,4'-dichlorodiphenyl sulphone (DCPS2) and 4,4'-dihydroxydiphenyl sulphone, also known as bisphenol S (BPS). Two simulants were used, i.e. 50% ethanol and 3% acetic acid solutions. The simulants used were that specified in the European Union Commission Regulation No. 10/2011 for milk and fruit juice respectively. The European Commission Directive 2002/72/EC has set the legislative migration limits for these chemical residues to be between 50–3000 µg/kg.

Keywords: *baby bottles, bisphenol A, bisphenol S, migration, food contact materials*

Acknowledgement: *C. Simoneau, L. Van den Eede & S. Valzacchi (2011): Identification and quantification of the migration of chemicals from plastic baby bottles used as substitutes for polycarbonate, Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment, DOI:10.1080/19440049.2011.644588*

N5
DETERMINATION OF CYCLIC OLIGOMERS OF POLYAMIDE 6 AND POLYAMIDE 66 BY MEANS OF LC–ESI–MS/MS IN FOOD SIMULANT B

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The various polyamides (PA) have a wide range of applications in the field of food contact materials. They are used on the one hand in kitchen utensils such as spoons or spatulas and on the other hand as foils in multilayer materials in the packaging of meat and cheese products (laminates) and as synthetic casings (sausages). The two most commonly used polyamides are PA6 (polycaprolactam) and PA66 (polymer of hexamethlyendiamine and adipic acid). In the course of polymerization/ polycondensation mostly cyclic oligomers are formed as reaction byproducts. The cyclic oligomers of polyamides belong to the so-called NIAS (non-intentionally added substances) which are being increasingly discussed in the frame of risk assessments. First findings showed that cyclic oligomers from polyamides could be transferred from food contact materials into foods or food simulants. In studies dealing with the migration of cyclic oligomers from PA6 and PA66 different analytical techniques have been used for their quantification in food or food simulants. HPLC–UV detection as well as application of LC–ESI–MS/MS for the sensitive determination of linear and cyclic oligomers of PA6 was reported. The National Reference Laboratory (NRL) for Food Contact Materials located in the Department of Chemical and Product Safety at the German Federal Institute for Risk Assessment has developed and validated a multi-oligomer analytical method for the identification and quantification of cyclic oligomers of PA6 (a total of eight) and PA66 (a total of four). Validation from the LC–ESI–MS/MS method for food simulant B (3% acetic acid) will be presented.

Keywords: *cyclic oligomers, Polyamide 6 (PA6), polyamide 66 (PA66), LC–ESI–MS/MS, food contact materials*

N6
ON THE KINETICS OF THE RELEASE OF ELEMENTS FROM CERAMIC FOOD CONTACT MATERIALS

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Ceramics for cooking and baking as well as for storing and serving food can be found in every household. The range of products includes earthenware, porcelain and stoneware. Surface finishes such as engobes, glazes and decorations add to the design of the products. However, coloured ceramic table and baking ware can contain heavy metals such as lead, cadmium and cobalt which may be released into foods. Amongst other factors, this is dependent on whether the ceramics were fired at high or low temperatures, on the type of food that was stored in the container and on the storage time. As a result of a reassessment carried out by the European Food Safety Authority (EFSA), new release limits for, at least, lead and cadmium for food contact with ceramic articles are foreseen in future European legislation. These will be significantly lower than the current limits. The European Commission has asked the European Union Reference Laboratory (EURL) and the National Reference Laboratories (EURL-NRL network) for Food Contact Materials to provide the scientific and technological basics and to establish new and robust methods for the testing of ceramic ware. The NRL for Food Contact Materials located in the Department of Chemical and Product Safety at the German Federal Institute for Risk Assessment is contributing to the development and harmonisation of test conditions for the release of elements from various ceramics. In daily use food contact materials made of ceramics are often used with hot foods. To investigate the influence of the foreseeable temperature conditions, the kinetics of the release of element ions from different food contact materials made of ceramics into tomato puree and the simulant 0.5% citric acid solution was examined in the temperature range of 25°C to 90°C. The release of the elements was tested to demonstrate its agreement with and predictability by the Arrhenius equation. The activation energies for the release of the individual elements were determined.

Keywords: *ceramic, food contact materials, kinetic, ICP–MS*

N7
CHEMICAL CONTAMINATION OF PAPER-BASED FOOD CONTACT MATERIALS

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Paper and paperboard have been reported to contain various chemicals including mineral oil aromatic hydrocarbons (MOAH), phthalates, photoinitiators, bisphenols and polyfluorinated compounds (PFC) either resulting from the manufacturing process or entering the final product unintentionally. When paper is used for food packaging, present contaminants can easily transfer into contacted foodstufs. Our study attempted to bring the mentioned 5 groups of contaminants under control using a recently developed multianalyte method based on a QuEChERS-like extraction followed by both GC–MS/MS and HPLC–MS/MS. Analysis of 68 target analytes in 132 commercially available paper packages revealed the presence of 65 analytes reaching levels up to 628 mg/kg. Especially MOAH, phthalates, photoinitiators and bisphenols occur frequently in complex mixtures resulting in a serious safety issue which makes paper-based food contact materials worthy of control.

Keywords: *paper, food contact materials, gas chromatography, high performance liquid chromatography, tandem mass spectrometry*

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N8
HEALTH SAFETY OF FOOD CONTACT PAPER EVALUATED BY IN VITRO TOXICOLOGICAL METHODS

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Paper and paperboard are widely used as food packaging materials intended for direct contact with foodstuffs and have to comply with a basic set of criteria concerning safety. A number of chemicals, such as slimicides, bleaching agents, and inks are used during the paper production process. Consequently, the total amounts of extractables might be high and the number of compounds considerable, including many unknowns. In the recent years a number of short-term bioassays focused on cytotoxicity and genotoxicity have been proposed as an approach for safety assessment of the chemically complex food contact paper materials and a draft methodology for biological testing has been published as the outcome of the joint European Commission/Industry project known as Biosafepaper. In our screening study we have tested extracts of 141 commercially available paper and paperboard samples in the 3T3 Balb/c NRU cytotoxicity test. Extracts of 75 samples (53.2%) induced cyto toxic effects in the cell culture. Selected samples with high cytotoxicity were further tested using reconstructed human tissues EpiIntestinal, EpiIntestinal FT (MatTek) and Colon epithelium (Sterlab) as models mimicking the human gastrointestinal tract. MTT viability assay was employed for evaluating the toxic effects of paper extracts in the tissues. HPLC–TOF–MS was used for analysis of the extracted compounds and for detection of their possible penetration into the culture medium underneath the tissues. ELISA method was employed for investigation of cytokine release suggesting inflammatory reactions. The results confirmed high sensitivity of the cytotoxicity test enabling to detect minor differences in the toxicity potential of paper samples. However, the study on reconstructed human models confirmed that even highly cytotoxic extracts did not affect the viability of complex human tissues despite exaggerated exposure.

Keywords: *paper, food contact materials, cytotoxicity, reconstructed human models*

Acknowledgement: *The research was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic (NT 14375-3/2013).*

N9
DETERMINATION OF BENZOPHENONE AND ITS DERIVATIVES IN DRIED FOODSTUFFS USING GC–EI–MS/MS

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Benzophenone and related derivatives are chemical substances present in our environment. Their chemical structure is based on two phenyl rings connected by a carboxyl group, with substitution groups such as methyl, hydroxyl or ethers. These compounds exhibit low molecular weights (<350 g/mol) and present hydrophobic properties. Because of their volatility, they exist in the atmosphere. Indeed, as some benzophenones are currently used by industries as photo-initiators in inks and UV filters in plastic packaging, they enter in the composition of food plastic packaging and cardboard boxes. The main human exposure sources have been identified through the consumption of contaminated food products and the use of cosmetics. Although their toxicity is low, it has been pointed out that benzophenones exert an effect on animals and humans. They are suspected to be endocrine disruptors and carcinogenic (Rodríguez-Gómez et al., 2015; Rhodes et al., 2007) with consequ ences in terms of human health which in turns may constitute an emerging chemical food safety issue. According to the European legislation, specific migration limits have been set for some of them (Reg 10/2011). In the past few years, several notifications from the EU Rapid Alert System for Food and Feed were related to benzophenone and methylbenzophenone, mostly in cardboard food contact materials. In the present work, an analytical method dedicated to the measurement of benzophenones directly in dried foodstuffs was developed. The target compounds were benzophenone (BP), 4-methylbenzophenone (MeBP), 4,4-difluorobenzophenone (FBP), 2,4-dihydroxybenzophenone (2,4-DHBP), 2-hydroxy-4-methoxybenzophenone (HMBP), 2,2'-dihydroxy-4-methoxybenzophenone (DHMBP), 2-hydroxy-4-n-octyloxybenzophenone (HOxBP) and 4,4'-dihydroxybenzophenone (4,4'-DHBP). The method involves gas chromatography coupled with a a triple quadrupole mass spectrometer operating in the electron impact mode (GC-EI-MS/MS, Scion TQ, Bruker) with a prior silylation step for separation and detection of target compounds. Extraction was performed by solid-liquid extraction using acetonitrile under ultrasonic conditions. Three deuterated internal standards (non, mono and di-hydroxylated) are used for quantification purpose, according to the isotopic dilution principle. The method was applied to the determination of the target compounds in several matrices (n=23) selected at a local distributor and representative of cardboard boxes packed food items. Besides proving the efficient management of analytical contamination, the implementation of the developed protocol enabled quantifying MeBP in food items at levels from a few ng/g to a few µg/g. BP and FBP were only quantified in one sample each at levels below 50 ng/g. The other target compounds were not detected.

Keywords: *food contact material, benzophenone, GC–MS/MS, methylbenzophenone, foodstuffs*

N10
CHARACTERIZATION OF MIGRANT COMPOUNDS FROM NEW ACTIVE MATERIALS FOR ITS APPLICATION IN FOOD PACKAGING OF FRESH FRUITS AND VEGETABLES

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The determination of additives and migration products from new active materials used in food contact materials is a challenge task in the agroalimentary sector. According to EU directive 2002/72, materials that come into contact with food products should be tested for possible leachables. To this end, the use of liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC–Q–TOF-MS) in combination with databases was used as powerful tools to identify migration products. The food packaging materials (PM) studied were plastic films based on polypropylene (PP), polylimonene (PL) and zinc oxide (ZnO) nanoparticles as antioxidant/antimicrobial agent in different percentages. Substances intended to be released from PM were checked determining released concentration per weight of article 3% (*w/w*) for ZnO nanoparticles. Migration tests also were useful for detection and characterization of non-intentionally added substances (NIAS) by accurate mass spectrometry toge ther with high MS/MS spectral acquisition.
Test conditions (temperature, time of contact and food simulant(s)) used for the packaging materials assays were selected according to EU Commission Regulation Nº 10/2011 and Directive 2002/72/CEE. Previous experiences have shown that a Tª control is essential to obtain repeatable results. For that, an incubator at Tª20ºC was used in this work to ensure thermostatic conditions during all tests. The chosen method consisted in the total immersion of the plastic materials intended to come in contact with foodstuffs into aqueous simulant(s), during 10 days as time of contact, taking into account the recommendations of the new Regulation and considering the worst case scenario according the real use of the packaging materials (contact with cooled food: salads or fruits). Plastic films based on PP, PL and Zn nanoparticles were tested in Mili-Q water 10% ethanol (*v/v*) (simulant A) and 3% acetic acid (simulant B). For that, plastic pieces (10×10 cm) were submerged in 50 mL of corresponding simulant inside glass test tubes. Three replicates of each migration test were performed. After that, the samples were analysed by accurate mass spectrometry (HRMS) and inductively coupled plasma mass spectrometry (ICP–MS).
Results and discussion
The migration test results have indicated:
• Five compounds were tentatively identified as migrating species under the experimental conditions with the simulants B, while only 3 with simulant A.
• The simulant B removes a greater amount of ZnO nanoparticles from the films.
• The addition of 5 wt% of PL to PPR hinders the migration of ZnO. In view of the initial results, it seems plausible to indicate that the ternary film PPR/PL/ZnO (92/5/3) is the more suitable polymer material for food packaging. The presence of PL is necessary to decrease the migration of ZnO nanoparticles, in comparison to that obtained with PPR/ZnO (97/3)

Keywords: *zinc oxide, food contact materials, migration, NIAS, Q-TOF.*

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N11
LC–MS/MS ANALYSIS OF PERFLUOROALKYL ACIDS IN FOOD AND FOOD PACKAGING MATERIAL – A MIGRATION STUDY

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PFAA are useful anthropogenic chemicals that are widely used in consumer and industrial products. This class of compounds includes thousands of chemicals, including perfluorocarboxylic acids, such as perfluorooctanoic acid (PFOA) and perfluorosulfonic acids, such as perfluorooctanesulfonic acid (PFOS). Many of these compounds are toxic, resistant to degradation, bioaccumulate in the food chain and they are regularly found in the blood of animals and humans worldwide. Due to their persistence the production of PFOA and PFOS was phased out in 2000 and other substances with similar chemical properties, like short chain PFFA, are used instead. PFAA are used to coat the surface of cookware and food packaging (non-stick coating) and can migrate into food, thus, represent a potential source of human exposure. In this paper we present a method using LC–MS/MS for the analysis in food and food packaging material to study migration. Samples were extracted using simple solvent extraction and analyzed using the SCIEX QTRAP® 4500 system in negative polarity electrospray ionization. Quantitation was performed with good linearity ($r > 0.99$) and repeatability (%CV < 10%). MRM ratios and QTRAP® MS/MS spectra were used for confident compound identification. Short chain perfluorocarboxylic acids ($C < 8$) were detected in different samples. We also observed the migration of PFBA from the cupcake wrapper into the cupcake.

Keywords: *perfluoroalkyl acids, LC-MS/MS, PFOA, PFOS, packaging material*

N12
QUANTITATIVE DETERMINATION OF 30 PLASTICIZERS IN FOOD PRODUCTS WITH LC–ESI–MS/MS

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Plasticizers like phthalates or adipates are widely used in plastics and packaging materials. Because of migration into the environment plasticizers can be found in food, feed, soil and even air samples. This ubiquitous contamination of plasticizers has become an important source of plasticizers in foods, in addition to direct migration from food packaging materials. It is well known that some phthalates, like di-2-ethylhexyl phthalate (DEHP), are suspected of being carcinogenic [1]. As most plasticizers are lipophilic they commonly can be found in fatty foods. Therefore a simple, sensitive and accurate method for analysis of plasticizers in food products is needed. In this study, a LC–ESI–MS/MS method for the quantitative determination of 30 plasticizers in food products is presented. For sample preparation, the homogenized food sample is extracted with acidified acetonitrile and a simple clean up procedure was also included. For LC separation of analytes a phenyl-hexyl reversed phase column was chosen. For MS measurement, the $[M+H]^+$ precursor ion was isolated for each analyte and two product ions were selected for data evaluation. Calculation was carried out using calibration standards of defined concentrations by correction with internal standards. Validation of the method showed good results for accuracy and precision. For matrices like vegetable oils, canned foods (vegetables, pesto) and beverages the values for coefficient of variation for precision were below 10%. The accuracy for nearly all analytes lay in the range of 80 to 120%. The LOQs for 26 analytes were 100 µg/kg, for the remaining 4 plasticizers they were 500 µg/kg. All FAPAS proficiency tests from 2014 to today were passed with z-scores ≤ 1,1 with the here presented method approach.

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Keywords: *plasticizers, phthalates, LC–MS/MS, UHPLC, food contaminants*

N13
DETERMINATION OF BADGE, BFDGE AND NOGE AS WELL AS THEIR DERIVATIVES IN FOOD USING UHPLC–ESI–MS/MS

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BADGE (bisphenol A diglycidyl ether), BFDGE (bisphenol F diglycidyl ether) and NOGE (novolac glycidyl ether) are starting substances for the polymerization of epoxy resins, which are commonly used for inner coatings of food cans. These epoxy-based lacquers, intended for protecting packed food from spoilage, pose a potential danger because monomers as well as oligomers can migrate from coatings into the food. Hydrolyzed derivatives can be produced during storage when the lacquer comes in contact with aqueous or acidic food. In addition chlorinated derivatives may occur during the thermal coating process. Because of suspected carcinogenic and genotoxic effects to humans specific migration limits are set in Commission Regulation (EC) No 1895/2005. The sum of migration of BADGE, BADGE H_2O and BADGE 2 H_2O into food or food simulants shall not exceed 9 mg/kg. The limit for the sum of BADGE HCl, BADGE 2 HCl and BADGE H_2O HCl is 1 mg/kg in food or food simulants. Be cause of the lack of toxicological evaluation the use of BFDGE and NOGE is forbidden for the production of materials which come in contact with food [1, 2]. In this poster a method is described which allows simultaneous detection of 13 substances of epoxy derivatives. Analytes are extracted from samples using methanol. Samples with complex matrices can be prepared with additional C18-SPE-clean up. An ammonium formate/acetonitrile gradient, acidified with formic acid, and a C18 endcapped column is used for separation. The developed UHPLC method is coupled with a triple quadrupole mass spectrometer and an ESI source in positive mode is applied for ionization. The $[M+NH_4]^+$ adducts serve as precursor ions for all analytes and three product ions are recorded for each analyte (one quantifier, two qualifiers). The evaluation of BADGE is carried out using calibration standards of defined concentrations by correction with an internal standard (D_6-BADGE). All other analytes are corrected via the external recovery of a spiked recovery sample. Validation of the method shows very good precision for all analytes in matrix-free sample solution (VK from 1 to 9%). Precision is also very good in matrix-matched sample solutions for BADGE (VK < 4%). Due to less precise correction by external recovery rate, the precision of the other analytes in matrix-matched samples is lower (VK from 5 to 19%). For validated matrices (fish from cans, fruit and vegetable from cans, fats and oils, beverages) the LOQ´s are less than 10 ppb for most analytes. For two chlorinated derivatives the LOQ´s are in the range of 100 ppb because of low sensitivity. Accuracy is very high for all analytes (in a range of 80 to 100%).

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Keywords: *BADGE, NOGE, UHPLC, LC–MS/MS, food contaminants*

N14
DETERMINATION OF BISPHENOLS IN HONEY

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Plastics are everywhere, and bisphenol A (BPA), a common ingredient in polycarbonate polymer and epoxy resins has become ubiquitous such that human and animal exposure to it is unavoidable with the principle route of human/animal ingestion via contaminated food. Confronted by tightening regulations, producers are turning to BPA alternatives (BPs), which are structural analogues of BPA. Recent studies indicate that some BPA alternatives also show estrogenic activity, while their presence in the environment and in food, e.g. honey, is only now beginning to be investigated. The aim of this study was to develop an analytical method for simultaneous analysis of BPs including BPA, BPAP, BPB, BPC, BPE, BPF, TMBPA, BPS, BPZ, DHBP and HPP in honey samples at trace levels (ng/g honey). The method involved dissolving 10 g of honey sample in 100 mL of water. The samples were filtered and extracted using SPE (Oasis HLB, 60 mg). The samples were derivatized with MTBSTFA with 1% TBDMCS in e thyl acetate and analysed by GC-MS. The method was found to be linear over the working concentration range (0.25–30.0 ng/g honey; R^2> 0.95) for all tested BPs. Average repeatability (as RSD; n=3) was 9.9%. The limits of detection (LODs) and limits of quantification (LOQs) were ≤0.548 ng/g honey and ≤1.83 ng/g honey for BPs, respectively. A series of honey samples were analysed from various European countries (e.g. Greece, Italy, and Slovenia), and China. BPA was present in all 6 samples (0.677–102 ng/g honey), while BPC was found in 3 samples (3.43–37.1 ng/g honey). Two samples contained BPE (2.35–8.32 ng/g honey), one sample contained BPF (8.32 ng/g honey) and one BPAP (3.21 ng/g honey). A honey from Italy contained the highest amount of BPs (BPA, BPE, BPC and BPAP). Based on the exposure from observed maximum levels of BPA in honey, we estimate that the consumer health risk is less likely (exposure less than TDI 4 µg/kg bw/day). The results suggest that BPA analogues or their mixtures are present in food (honey), which means that cumulative health effects of these substances should be explored in the future. Further work will include the analysis of more honey samples and the search for the origin of contamination with the detected BPs (e.g. food contact materials).

Keywords: *bisphenol, honey, organic contaminants, food contact materials*

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N15
FOOD CONTACT MATERIAL (FCM) MIGRATION STUDY USING HR–LCMS

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To ensure consumer safety, food/beverage packaging companies are required to conduct Food Contact Material (FCM) migration studies following regulatory guidelines. FCM migration study is the key component of marketing approval document "Food Contact Notification (FCN)" by authorities around the world. FCM migration study requires components identification, characterization, and quantitation for safety assessment. Because of sample complexity and unknown, unexpected nature of some components, advanced analytical instruments, combined with good software and database can significantly alleviate the challenge of FCM migration study. This study presents a workflow for food packaging bags, both non-gamma-irradiated and gamma-irradiated, migration study using HR-LCMS, GCMS, and database search. Bags were extracted using 3% acetic acid and 50% ethanol food simulants following FDA guidance. Samples were chromatographically separated by a gradient using an Ultimate 3000 RSLC HPLC and HRAM MS analysis was conducted on Thermo Q Exactive Plus mass spectrometer. HRAM full scan MS and data-dependent HCD MS/MS data were collected with polarity switching using resolution 70,000 and 150,000 respectively. The data was processed using Thermo software SIEVE™ and Mass Frontier™, searched against ChemSpider™ and mzCloud™ spectral database. Quantitation was conducted using SIM scan at resolution 70,000. GCMS analysis was carried out on Thermo Trace Ultra GC and ISQ MS. The compounds were identified by NIST library. The HRAM full scan data allow confident component identification and elemental composition assignment. The information-rich HCD MS/MS fragments provide valuable data for structure elucidation. Rapid positive/negative polarity switching gives additional information and confidence in component detection and characterization. The HRAM data was processed using SIEVE software for differential analysis. The identified components were s earched against ChemSpider and mzCloud spectral database for known compound identification and structure characterization. The putative structures of unknown components were proposed based on HCD MS/MS fragments; the proposed structures were searched against the HighChem fragment and mechanism library by using its "Fragment and Mechanism" feature. The high resolution quantitation was carried out using SIM scan at resolution 70,000. The antioxidant Irganox1035 was selected as model compound. The calibration curve ranged from 0.05 ppb to 100 ppb, using linear regression and 1/x weighting. The result demonstrated great linearity precision, accuracy, and sensitivity with excellent LOQ. GCMS analysis provided complementary information for volatile and semi-volatile components of the samples. The preliminary result of this study demonstrates an effective workflow for food contact material migration analysis by using high resolution mass spectrometer, in combination with novel s oftware and database suite.

Keywords: *library searching, migration, packaging, unknown contaminates*

PROCESSING CONTAMINANTS

(O1 – P10)

O1
DETERMINATION OF ETHYL CARBAMATE CONTENT IN SWEET SHERRY WINES BY HPLC–FLD

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Ethyl carbamate is a known carcinogen that has been found mainly as a byproduct of fermentation, through which it is formed from various precursors, notably urea and citrulline [1], in a reaction promoted by heat. Although maximum allowable levels of EC for different kinds of wines were established by Canada or USA, there are currently no harmonised maximum levels for ethyl carbamate in the European Union (EU) [2]. In Andalusia (Spain), special sweet wines are obtained using dried grapes by direct exposition to the sun. Muscat and Pedro Ximenez grapes, in bunches, are spread out on esparto grass mats to toast in the sun before pressing. After that, the must obtained is in part fermented and fortified up 17% with alcohol. Finally, wines are aged in oak casks which, in the case of Jerez region, have been previously used for Sherry wines. Fortification process may aggravate the problem of urea excretion by yeast. Urea is often formed during the early and middle stages of fermentation with subsequent yeast generations utilizing it during the later stages. If the fermentation is early stopped by fortification, wines with higher urea content can be obtained [3]. In this study, several experiments of aging in oak wood and stainless steel of sweet Sherry wines obtained from grapes cv. Muscat and Pedro Ximenez (vintages 2008, 2009 and 2010) and fermented under different enological conditions have been carried out. These sweet wines were periodically sampled and ethyl carbamate content was analyzed using HPLC with fluorescence detection after derivatization with 9-xanthydrol. The method was linear over the range from 10.0 to 350.0 μgL^{-1} of EC, with a regression coefficient of 0.9992.The results suggest an important influence of the different factors considered (vinification conditions, aging type, aging time, grape variety and vintage) on ethyl carbamate content in the wines.

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Keywords: *ethyl carbamate, oak cask, stainless steel, sweet sherry wine, yeast strain*

O2
DEVELOPMENT OF STIR BAR SORPTIVE EXTRACTION METHOD FOR THE DETERMINATION OF VOLATILE COMPOUNDS IN ORANGE JUICES

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Orange is one of the most produced fruit all over the world and it is cultivated in the five continents. Brazil is the leading producer country and Spain is the sixth producer worldwide and the first in Europe with 3.4 millions of tons in 2013. Its aroma makes it a really attractive fruit for daily consumption, especially as a juice, so the study of this aroma is very important because it is closely related to product acceptance. The principal volatile compound found in orange juice is limonene, a monoterpene, which concentration is up to 40–90% of the total concentration of all volatile compounds. However, there are many families of volatile compounds that contribute to the global aroma of oranges such as aldehydes, aliphatic alcohols, carboxilic acids, esters, ketones monoterpenes, monoterpenes alcohols, and sesquiterpenes. Therefore, it is interesting to set up analytical methodology for the analysis of this kind of compounds. A stir bar sorptive extraction (SBSE) method for the determination of volatile compounds in orange juices was developed. The extraction variables were optimized using a reduced two-level factorial design, and the most suitable analytical conditions for the extraction of the studied compounds were: sample volume 10 mL, extraction time 60 min, stirring speed 1800 rpm, NaCl amount 30 %, and twister length 10 mm. The optimized method was further validated, obtaining good linearity ($r^2 > 0.99$) and detection and quantitation limits low enough to determine correctly the studied compounds. As well, for most of the studied compounds precision and recovery values were acceptable (values under 15% and over 60%, respectively). Several orange juice samples (squeeze and commercial) were extracted with the optimized extraction method and analyzed by GC–MS. The method has proven to be suitable enough for the determination of the aroma of orange juice, being limonene the major volatile comp ound in all the studied samples.

Keywords: *orange juice, volatile compounds, SBSE–GC, factorial design, optimization*

O3
DETECTION AND EFFECTS OF MIXTURES OF HEAVY POLYCYCLIC AROMATIC HYDROCARBONS IN ZEBRAFISH EMBRYOS

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Polycyclic aromatic hydrocarbons (PAHs) are among the most widespread and most toxic pollutants formed by the incomplete combustion of fossil fuels and organic materials originating from natural and anthropogenic sources. Furthermore, some food processing techniques such as grilling, barbecuing and smoking are responsible for the production of PAHs in foodstuff such as fish and meat. In nature, PAHs are usually found as mixtures and represent an important health concern since they have been found to be carcinogenic, mutagenic, genotoxic and immunotoxic to a variety of organisms including human. In spite of their acknowledged adverse impact on human and environmental health, the toxicity evaluation of such compounds is still troublesome. In fact, PAHs are well known for having a very low aqueous solubility additionally they are prone to undergo photodegradation processes. Moreover, due to their high hydrophobicity and solid-water distribution ratios, they ten d to interact with non-aqueous phases and organic matter and, as a consequence, they become potentially unavailable to testing organisms. To overcome these problems, in this work, we developed a protocol to detect and test in a realistic and effective way the toxic effects and the bioaccumulation factor of a mixture of 5 heavy PAHs in the zebrafish animal model. Solubilisation and stabilization of the compounds was achieved by supplementing the exposure media with dimethyl sulfoxide (DMSO) and Tween 20. Exposure of 6 h in the absence of light were sufficient to detect toxic effects in zebrafish embryos by evaluation of the differential expression of some selected genes related to the Aryl Hydrocarbon Receptor (AHR) gene battery. Although the bioaccumulation of parent PAHs was negligible, the results obtained revealed the induction of several genes, in particular cyp1a1, even at concentrations of exposure below the MRL established by the EU, confirming that PAHs suffer very rapid metabolisation inside the embryos, and that those biotransformation products yield changes on the expression of some genes relevant for PAHs toxicity. The interaction between five different PAHs in a complex mixture was evaluated and the results obtained demonstrate the presence of synergistic effects on the regulation of the genes studied. As a consequence, complex mixtures of PAHs (concentrations and combinations close to reality) should be evaluated to get an accurate view of the potential adverse effects of this kind of compounds and allow the performance of a reliable toxicological risk assessment. The new developed protocol can also be applied to the detection of residues of other contaminants and therefore, be used as a tool for evaluating potential risks in environmental and food samples.

Keywords: *polycyclic aromatic hydrocarbons, toxicity evaluation, zebrafish, mixture toxicity*

Acknowledgement: *Spanish Ministry of Economy and Competitiveness (SAFEFOOD project, grant code, CTQ2014-54801-C2-2-R)*

O4
ANALYSIS OF QUATERNARY AMMONIUM COMPOUNDS (QACS) AS POSSIBLE DISINFECTANT RESIDUES IN MILK BY LC–TOF

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Quaternary ammonium compounds (QACs) possessing R groups with long alkyl chains are known to be especially effective as antimicrobial agents and particularly useful for the disinfection of containers and surfaces. This is particularly relevant in the milk industry, as QACs are typically used to disinfect the insides of tanks used for transporting milk from farms to processing plants. If significant QAC residues are left behind after disinfection, these compounds may get into the processed milk and, ultimately, may get into the store-bought milk supplies at levels compromising personal health. Recent data points to nearly 12% of all monitored milk to be tainted with QACs [1]. Considering the above, this application note presents an LC–TOF (time-of-flight) method for the analysis of the four most common QACs that may be found in milk with relatively little sample preparation. This method takes advantage of the inherent mass accuracy and high resolution afforded by TOF detection for specificity and component identification. Method conditions and performance data, including linearity and repeatability, are presented.

[1] http://www.efsa.europa.eu/de/supporting/doc/483e.pdf

Keywords: *milk, disinfectant residues, LC–TOF*

O5
UNTARGETED DETECTION OF CONTAMINANTS IN AGRO-FOOD PRODUCTS USING VIBRATIONAL SPECTROSCOPY AND CHEMOMETRICS: THE EXAMPLE OF DETECTION OF MELAMINE LEVELS IN MILK

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In this study, a procedure is proposed for the characterization of agro-food products using vibrational spectroscopic analysis tools such as mid-infrared (MIR). The objective is to exploit the huge amount of information contained in the data generated by such techniques, which could support the concept of data-driven discovery or untargeted analysis. New crises of adulteration/contamination with illegal ingredients other than known ones continue to occur from time to time. By relying only on targeted analysis methods, adulteration could get out of control and analysis would become trapped in a cycle of 'adulteration, targeted analysis, and new adulteration', and so on. In contrast to targeted analysis, which uses information from known possible unusual ingredients, an untargeted experiment registers all information within a certain correlation/similarity, including data from new products. Untargeted detection methods are therefore required for screening products for a range of known and unknown adulterants. Untargeted analysis will mean alerts can be given more rapidly and fraud detected more easily. Until now, untargeted analysis has been associated mainly with direct analysis techniques, such as mass spectrometric-based metabolomics or isotope-assisted methods. Only a few studies have linked untargeted analysis with vibrational spectroscopic methods. In this study, vibrational spectroscopic techniques combines with new concepts in multivariate analysis s for characterizing liquid UHT milk samples spiked with varying levels of melamine. Melamine has been illegally added to food/feed to artificially elevate the protein content value of products. Since the discovery of melamine contamination in infant milk formula in China, strict regulations have been enforced throughout the world and many papers have been published on the use of such methods as wet chemistry, chromatography, mass spectrometry and vibrational spectroscopy to detect melamine in both raw and powdered milk. In this study, liquid ultra-high temperature (UHT) milk was contaminated with melamine at various levels ranging from 0.01% to 1% (100–10,000 ppm) and measured using Fourier Transform Mid-Infrared (FT–MIR) spectrometry in order to test the performance of the new chemometric method and determine its limits of detection.

Keywords: *vibrational spectroscopy, contamination, melamine, milk, chemometrics*

Acknowledgement: *This work was performed in the framework of the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 613688 FOODINTEGRITY project.*

O6
DEGRADATION OF PESTICIDES IN AQUEOUS SOLUTION BY UV AND UV/H_2O_2 TREATMENTS

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Pesticides are often reported in aquatic environmental contamination as being a result of soil leaching and improper disposal of agricultural packages. Advanced oxidation processes have been studied as alternatives to treat such compounds in aqueous media. Degradation of five pesticides (cyazofamide, fenhexamid, kresoxim-methyl, metrafenone, and ametoctradin), was investigated in aqueous solution using ultraviolet light (UV) and a combination of ultraviolet light and hydrogen peroxide (Xe arc lamp and H_2O_2). Photochemical experiments based at two-level with three control factors: pH (2.0, 6.2 and 12.0) and H_2O_2 (1, 50 and 100 $mg.L^{-1}$), were conducted. The pesticides were analyzed using ultra high performance liquid chromatography with mass spectrometry (UPLC–MS). Under the best conditions, 98% of kresoxim-methyl, 93% of fenhexamide, 97% of ametoctradin and 82% of metrafenone, were removed in about 240 min, and 98% for cyazofamide in 15 min. The study also showed that the oxidation rate was enhanced during the UV/H_2O_2 process in comparison to direct photolysis. The results of the study suggested that the concentration of 100 $mg.L^{-1}$ H_2O_2 and pH 2 with 240 min UV irradiation time were the best conditions for a faster photochemical degradation of the pesticides. The photochemical degradation with UV/ H_2O_2 can be an efficient method to remove the pesticides from aqueous solution.

Keywords: *photooxidation, UPLC–MS, hydrogen peroxide, pesticides, photoproducts*

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O7
CONCENTRATIONS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED FOODS IN JAPAN

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Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic compounds consisting of two or more fused aromatic rings. PAHs are formed mainly during cooking and during processes such as smoking. There are currently 16 PAHs that are considered to be of health concern by the EU (EU priority PAHs), but there has been only a limited number of studies of the concentrations of these 16 PAHs in smoked foods in Japan. Here, we developed and validated a GC–MS/MS method for analysis of the 16 PAHs and used it to determine their concentrations in smoked foods. A total of 76 samples of smoked fish (n=20), smoked meat (n=16), smoked eggs (n=10), dried bonito flakes (n=10), and dried bonito–related soup-stock products [disposable powder packets for infusion (n=10) and liquids (n=10)] were purchased for analyses of the 16 PAHs on the Japanese market in 2012–2014. Note that the analytical values for some PAHs (e.g. benzo[c]fluorene, chrysene, and dibenzo[a,h]pyrene) are given for information only because of low certainty of the results. The highest median concentrations of benzo[a]pyrene and the highest sum concentrations of all 16 PAHs were found in the disposable powder packets (29 and 760 µg/kg, respectively), followed by dried bonito flakes (24 and 510 µg/kg, respectively), although the sum concentration of the 16 PAHs was likely overestimated, mainly because of incomplete separation of chrysene and triphenylene in the GC column. The median concentrations of benzo[a]pyrene and the sum concentrations of the 16 PAHs in the other food categories were much lower than in the abovementioned categories. Benzo[c]fluorine, benzo[a]anthracene, cyclopenta[c,d] pyrene, and chrysene—the molecular weights of which are relatively low among those of the 16 PAHs—were detected at high concentrations in most of the samples. Furthermore, we investigated the transfer rates of the 16 PAHs from dried bonito flakes and one of the related soup-stock products (disposable powder packets) to hot water, because these products are used mostly for making soup stocks. The transfer percentages of PAHs from the tested products to commonly prepared home soup stocks were extremely low (not exceeding 3%), even these test products contained relatively high concentrations of the 16 PAHs. This finding could be explained by the low solubility of the 16 PAHs in water.

Keywords: *polycyclic aromatic hydrocarbons, smoked foods, GC–MS/MS, dried bonito, Japan*

Acknowledgement: *This work was supported by a Health Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan.*

O8
QUANTIFICATION OF HISTAMINE IN SELECTED CHEESES BY HILIC–MS/MS

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Histamine is one of the main biogenic amines that are usually found in the scombrids fish family such as tunas, mackerels and bonitos. However, this pseudo-allergen may also occur in cheeses during storage or ripening. As the Swiss consume typically more than 21 kg of cheese per person (2013 data), the present study aimed at monitoring the histamine content in this highly consumed dairy product to evaluate potential consumer exposition. For this purpose, in the course of 2014 our laboratory planned to conduct a large survey on histamine analysis on medium-hard, hard and extra-hard Swiss cheeses. For comparison, a number of widespread and popular foreign cheeses in Switzerland were also analyzed. Milk processing and ripening time were also considered. In total 301 samples were analyzed. The polar histamine was quantified without any derivatisation step under HILIC conditions and detected by MS/MS by following two specific mass transitions. Amongst all samples, 88% of them contained less than 200 mg/kg of histamine while 8% were between 200 and 500 mg/kg. Only 4% exceeded the value of 500 mg/kg with up to 1016 mg/kg for an Italian cheese. These results showed that most cheeses contain irrelevant concentration levels of histamine and that maximum levels measured do not reach those of highly contaminated fish samples. Currently the EC and Switzerland have set a maximum limit for histamine in fish (100 mg/kg), but not for cheese where some observed amounts could yet cause some health concerns. A full report was submitted to the Federal Office of Food Safety to establish the risk assessment and possibly regulate maximum levels of histamine authorized in cheese.

Keywords: *histamine, food control, HILIC–MS/MS*

O9
LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS) DETECTION OF GLYCIDYL ESTERS AND MCPD ESTERS IN INFANT FORMULA

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Fatty acid esters of 3-chloro-1,2-propanediol (3-MCPD), 2-chloro-1,3-propane diol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. Formed during the deodorization of oils during refining, these compounds are considered potentially carcinogenic and/or genotoxic, making their presence in edible oils and processed foods containing these oils a potential health risk. Recently, there has been increasing attention to the use of these refined oils in commercial infant formulas. Since formula is typically an infant's sole source of nutrition, and due to infant's low body weights, the presence of MCPD and glycidyl esters poses a potential health risk. At present, published validated methods for extracting and quantifying these contaminants in processed foods are limited to mayonnaise and salad dressings. The current work focuses on developing methodology for extracting and quantifying these chemica l contaminants from commercial infant formulas in order to determine levels of exposure. A method for extracting MCPD and glycidyl esters from infant formula will be described in this presentation. The extraction efficiencies of MCPD monoesters, MCPD diesters and glycidyl esters in a homemade infant formula with known MCPD/glycidyl ester concentrations will also be discussed. Results indicate that extraction efficiencies of greater than 90% can be achieved for all monoester, diester, and glycidyl ester species using the developed extraction procedure. Quantitation of MCPD and glycidyl esters was performed using a liquid chromatography tandem mass spectrometry (LC–MS/MS) method that was previously developed for the quantitation of these species in edible oils. Validation results indicate the extraction and quantitation methods developed for infant formula are sensitive and robust. Using the validated methodology, a survey of a number of commercially available infant formulas (in both the United States and abroad) was conducted in order to assess infant exposure to MCPD and glycidyl esters.

Keywords: *processing contaminants, MCPD esters, glycidyl esters, LC–MS/MS, infant formula*

O10
IMPROVED DNA-BASED DETECTION OF FOODBORNE PATHOGENS USING ENTEROHEMORRHAGIC E. COLI AS A MODEL

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Despite recent advances in food pathogen detection, many challenges still exist allowing for further improvements e.g. (i) sample processing and DNA extraction; (ii) implementation of multi-detection approaches and optimal use of the whole genome sequence has yet to be realized for improved and comprehensive risk assessment, and (iii) improved on-site detection tools. Currently, detection methods in food bacteriology involve at least one enrichment step that may include different selective agents, cultivation time and temperature as well as atmosphere combinations. Therefore, one of the great challenges is to reduce or optimize these enrichment steps and to optimize DNA extraction while retaining the sensitivity of the pathogen detection. To achieve this, large sample sizes are preferable which requires fully optimized DNA extraction protocols to compensate for the often low number of food pathogens present and their heterogeneous distribution in the food matrix. To tackle these questions, we use enterohemorrhagic E. coli (EHEC) as a model system and aim at optimizing sample preparation and DNA extraction approaches for detection and typing of EHEC from different food matrices such as meat, fresh produce and water. Today's methods for EHEC detection from food and environmental samples relies on enrichment, screening for at least stx-genes, and also often eae-genes and the top associated serotypes, followed by attempts of isolation in order to achieve an isolate that can be further characterized. Sample size is usually 25 g, but other sample volumes can be used. A recurring problem is the low isolation rates from PCR positive samples with the challenges this poses for food producers and competent authorities. One of the aims of Decathlon Work package 3 (Improved detection of pathogens) is to develop new, sensitive approaches based on PCR and NGS. So far, we have extended the number of LAMP assays to discriminate between the main discriminative EHEC markers stx1 and stx2. These fast and simple methods, that are easy to perform outside of laboratories using small and portable apparatus, can significantly improve the identification of the EHEC strains involved. We perform whole genome sequence comparisons of EHEC and related enterobacteria in order to identify discriminative markers for EHEC detection and subtyping. The wealth of genome sequence data available for E. coli has been used for comparative genomic analyses of EHEC, with the aim to identify novel discriminative biomarkers for improved EHEC detection. These results will be translated into the development of new, sensitive PCR- or NGS-based approaches for EHEC detection and characterization. Our results and the approaches developed are transferable and can improve detection and typing tools for other food pathogens and other harmful organisms.

Keywords: *foodborne pathogens, enterohemorrhagic escherichia coli (EHEC), comparative genomics, LAMP, PCR*

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RESIDUES

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PESTICIDES

(P1 – P75)

P1
SCREENING METHOD VALIDATION OF PESTICIDE RESIDUES IN CEREALS USING GC QTOF AND EXACT MASS LIBRARY

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The EURL for pesticide residues in Cereals and Feeding stuff in Copenhagen (EURL–CF) has develop screening analysis methods on both LC- and GC–QTOF instruments for pesticide residues. Analysis with GC–QTOF shows other challenges than with LC–QTOF, especially because the molecular ions typically don't survive the ionisation. The compounds are fragmented in the external ion source and currently no libraries with exact masses and full information on element composition exist. The EURL–CF is in cooperation with the EURL for pesticide residues in Fruits and Vegetables creating a database with exact masses of fragments for GC amendable pesticides.
The development of the screening method consisted of two parts. Firstly, the exact masses of fragments were determined and secondly validation experiments have been analysed and evaluated. The identification of the correct mass formulas for the ion fragments was done using general knowledge of fragmentation mechanism assisted by e.g. MS Interpreter in NIST Mass Spectral Search Program or Agilent Mass Hunter software. The calculated fragment masses were verified by measured masses of analysed pesticide standards. After determining the exact masses, these have been used to identify pesticides in validation experiments. The screening method is based on the QuEChERS method for extraction and clean-up. Validation experiments have been performed according to SANCO 12495/2012 where at least 95% of minimum 20 samples should be detected. Barley, oat, rice, rye and wheat cereal samples were spiked with pesticide standards, extracted and analysed by GC-QTOF. At least 20 samples consisting of 5–6 samples of each cereal type were spiked at levels of 0.01, 0.02, 0.05 and/or 0.1 mg/kg with different standard mixtures, containing around 200 different compounds per experiment. The analytes included were both commonly occurring pesticides and scarcely found pesticides. In addition to spiked samples, blank cereal samples and 4 EURL proficiency test materials were analysed. Initial validation results showed that 36 of the 38 evaluated compounds were validated and out of which 19 with Screening Detection Limit, SDL at 0.05 mg/kg and 17 with SDL 0.01 mg/kg. The identification criteria included mass accuracy of two diagnostic ions at ≤5 ppm for amu >200 and ≤1 Dalton for amu ≤200 in combination with the relevant retention time, Signal/Noise >6 and ion ratios <30%. All data processing was done by Mass Hunter Quantitative software. Analyses of the 4 EUPT test materials that contained 46 residues of 27 different pesticides in the range of 0.012–2.180 mg/kg showed very good agreement with the validation as all residues about the SDL were detected (45 residues). No false positives were seen. Additionally, rice samples from the Danish pesticide control programme were analysed. Identified residues have been confirmed with quantitative methods. Results from this and further validation data for more than 50 pesticides will be presented.

Keywords: *pesticide residues, GC–QTOF, exact masses, screening method, validation*

P2
A FAST, QUANTITATIVE AND QUALITATIVE QUECHERS BASED COMMODITY INDEPENDENT MULTI RESIDUE ANALYSIS ON A APGC–XEVO TQ-S (MICRO)

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A fast Quantitative and Confirmative Multi Residue Pesticide on a GCMS in differrent commodities in one generic method is often a challenge. At Nofalab we developed a commodity independent method for over 300 pesticides on a XEVO TQ-S in combination with a APGC. At Nofalab we employ the XEVO TQ-S in combination with a APGC (Atmospheric Pressure Gas Chromatography) already for over four years successfully. We developed a standard extraction method for all food and feed commodities based on QUECHERS. Using the benefits of the soft ionisation of an Atmospheric Pressure Chemical Ionisation technic (APCI) such as the APGC where M+ and MH+ ions are abundant with minor fragmentation. A high sensitivity is reached by using this ionisation technic. Defining MRM's on EI-ionisation is often based on fragments which are not specific for one compound. In contrast to EI-technics with APCI MRM's can be based upon the M+ and/or MH+ ions giving a better specificity. Based upon this excellent performance gave the opportunity to modify the QUECHERS-extraction in such a way that a stable chromatographic separation is obtained and matrix interference is minimized. This results in a commodity independent method for the determination of pesticides. Using a XEVO TQ-S this method was validated for 300 pesticides in a 30 minute injection to injection method. Recently Nofalab acquired a XEVO TQ-S Micro. Compared with the TQ-S this instrument has a significantly faster scanning rate. In ESI-mode the sensitivity of the instrument is significantly lower, but is APGC-mode the sensitivity is comparable since in contrast to the ESI all the effluent of the GC-column is extracted into the Mass Spectrometer. In this presentation the optimization of the APGC is discussed based on 25 pesticides reflecting several behaviours such as injection stability, resolution, M+, MH+ and fragment formation on a XEVO TQ-S and the new XEVO TQ-S micro. The presentation concludes with the results of a validation of a quantitative and qualitative method for over 500 pesticides (all with 2 or more MRM's) on à XEVO TQ-S Micro within à 30 minute injection to injection run. For most pesticides the LOD is less than one fifth of the required MRL's are reached, for some even lower than 0.001 mg/kg with acceptable recoveries of 70–120% and a RSD below 20% as required by SANCO.

Keywords: *APCI, Quechers, Nofalab, TQ-S Micro, APGC*

P3
FROM THE LEMON SURFACE UNTIL THE BAKED FOOD PRODUCT: QUANTITATIVE MONITORING OF THE PATHWAY OF IMAZALIL

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Imazalil is one of the most widespread fungicides used for the post-harvest treatment of citrus species and it is therefore localized mostly on the surface of fruits. As citrus peel is often used as a spice for household-level food processing and preparation, the carry-over of imazalil from citrus fruits to food products is an obvious and frequent event. Taking into account the relatively high stability of imazalil, the residual amount of this fungicide may not be negligible. The goal of our comprehensive study was to quantitatively monitor the pathway of imazalil throughout a complete food preparation event. That is, imazalil-treated lemon batches were purchased. After determining separately the imazalil contents of lemon peels and pulps, lemon peels were used to prepare a high-fat matrix model cake that went through a usual baking /200°C/ step, which models more closely the real household situation than spiking any food product with the standard solution of imazalil and avoiding any heat treatments to facilitate sample preparation issues. In order to extract imazalil quantitatively from the baked fatty cake matrix, the sample preparation approach was adapted and validated on the basis of the EN 15662:2008 and the AOAC 2007.1 methods with three modifications, i.e., (i) hexane was added to the extraction solution, (ii) the cleaning step was applied after an overnight freeze-out step; (iii) the C18 sorbent was added at the cleaning step of the sample preparation. Imazalil was quantified with the help of an HPLC–ESI–QQQ–MS setup, while imazalil degradation was followed with an HPLC–ESI–QTOF–MS system. As a conclusion, EN 15662:2008 method and its modifications generally resulted in better recovery values, mostly exceeding 90%, which meets the actual DG SANCO recommendations, if the freezing step was included in the sample preparation process. The degradation of imazalil during the baking process was significantly higher when this analyte was spiked to the cake matrix in standard solution form than in the case of preparing the cake with imazalil-containing lemon peel (52% vs. 22%). This observation calls the attention to the careful evaluation of pesticide stability data that are based on solution spiking experiments. Taking into consideration the washing procedures of the entire lemon samples as well, the overall carry-over rate of imazalil from the lemon until the baked cake products ranged between 55-76%. However there is no regulation in the EU for pesticide residues in final food products except for some special commodities such as baby foods, this high level of residual imazalil should trigger more real world scenario based experiments in this field.

Keywords: *imazalil, degradation, recovery, fatty matrix*

P4
DEVELOPMENT OF HPLC-MS METHODS FOR IDENTIFICATION OF TRIAZOL FUNGICIDE METABOLITES IN FRUITS AND VEGETABLES

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However a number of triazol fungicides are still frequently used for plant protection in the EU, several work have recently been revealed their carcinogenic effect for mammals. Although residues of these toxic compounds are strictly controlled before food consumption, these systemic agents are easily metabolised by plant tissues, avoiding the detection of these transformation products. On the other hand the toxicity and the mechanism of action of these structurally similar metabolites are unknown, but identification of these metabolites are essentialpart of the food safety. In our work HPLC–QQQ–MS method was developed for the detection of triazol fungicide metabolites in routine analyses of fruits and vegetables. In the first step of the work fragmentation behaviour of cyproconazole, difenoconazole, penconazole, tetraconazole, fluquiconazole, propiconazole, protioconazole, tebuconazole, myclobutanil, triadimenol, flutriafol, metconazole, epoxiconazole, fenbuconazole, bromuconazole was studied by HPLC–Q-TOF–MS system using different ion-source voltage and collision energy. Together 19 typical fragment ions were selected in order to detect triazol fungicides as well as their transformation products. For routine analytical purpose HPLC–QQQ–MS method in precursor ion scan mode was developed monitoring the diagnostic fragment ions together with their molecule originated. Distinction of parent compounds from their metabolites was carried out based on retention time of chromatographic peaks of the same diagnostic fragment ions. Identification of meta bolites was achieved by HPLC–Q-TOF–MS using (i) exact mass calculation, (ii) fragmentation pattern as well as (iii) isotopic distribution of the metabolite. By the use of the developed method tebuconazole-hydroxide was successfully identified in a routinely analysed grape sample.

Keywords: *triazol fungicides, HPLC–QQQ–MS, transformation products, diagnostic fragment ions*

P5
DETERMINATION OF NEONICOTINOIDS IN HONEY WITH LIQUID CHROMATOGRAPHY

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After the European Union temporary banned the use of the neonicotinoids in flowering crops that honeybee might visit, there has been an increased interest on determination of the neonicotinoids residues in honeybee products such as honey. Neonicotinoids are relatively new class of the insecticides chemically related to nicotine, and in the less than twenty years, neonicotinoids become the most widely used class of insecticides in the world. This group of the insecticides includes nitro-subsituted (dinotefuran, nitenpyram, thiamethoxam, imidacloprid and clothianidin) and cyano-substituited (acetamiprid and thiacloprid) compounds. The objective of this study was to develop analytical methods based on liquid chromatography, equipped with the two different detectors (diode array detector (DAD) and tandem mass spectrometry (MS/MS)) for the determination of the selected neonicotinoids. Sample preparation procedure for the analysis of selected neonicotinoids in hon ey samples included two different techniques: dispersive liquid–liquid micro extraction (DLLME) and QuEChERS commercial kit for pesticides sample preparation. The liquid chromatographic conditions were optimized by Response surface methodology with Box–Behnken design and the global Derringer´s desirability. Both of the optimized methods ware validated to fulfil the requirements of SANCO/12571/2013 standard for the sample pretreatment procedure providing results for accuracy (R, 70–120%), repeatability (RSD, <20%) and within-laboratory reproducibility (RSD, <20%), limit of detection (LOD, 2.5–5.0 μg kg^{-1}) and quantification (LOQ, 7.5–10.0 μg kg^{-1}). Matrix effects were compensated by the use of matrix-matched calibration. For the first time, more than 100 honey samples with different plant origin (sunflower, linden, acacia, wildflower) collected from all 7 counties of Autonomous Province of Vojvodina were analysed discovering the presence of selected neonicotinoids, therefore implicating the necessity of ongoing control of this type of food.

Keywords: *neonicotinoids, liquid chromatography, pesticides, honey, food contaminants*

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P6
PESTICIDES RESIDUES DETERMINATION IN FRUIT JAMS USING μLC–ESI–QTRAP–MS/MS

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Fruit jams originated as an effort to preserve fruits for consumption during the fruit off-season. It is an intermediate food moisture prepared by boiling fruit pulp with sugar, pectin, acid and other ingredients (preservatives, coloring and flavoring substances) until obtaining a reasonably thick consistency. Whereas that fruits frequently get pesticides application during its growing or even after harvest it is quite possible that fruit jams would show pesticides residues occurrence [1]. Thereby, this study aimed to develop and validate a selective, robust and highly sensitive μLC–ESI–QTRAP–MS/MS method to determine pesticides residues in fruit jams and apply it to the analysis of samples to verify the presence of pesticides in these commodities. Before the application of the QuEChERS extraction procedure, the fruit jams were slurried with ultrapure water (ratio 1:1.5, m/m) to yield homogeneous samples and to facilitate the sample handling. Because of the high sensitivity achieved with the μLC–ESI–QTRAP–MS/MS equipment and to minimize matrix effect, the acetonitrile extracts were diluted 30–fold, with acetonitrile/water (1:9, *v/v*) before the chromatographic analysis. The method validation was done by the analysis of spiked samples at the concentrations of 9 and 45 μg/kg, with 5 replicates each concentration. The method met validation criteria of 70–120% recovery and RSD ≤ 20% for 92% of the 107 evaluated pesticides. The reporting limit (RL) was 9 and 45 μg/kg for respectively 66% and 26% of the analytes, 5% of the compounds did not fulfill the requirements for validation and 3% were not detected at the studied concentrations. The matrix effect was calculated for each analyte comparing the slope of the calibration curve prepared in blank grape jam extract and in acetonitrile and was < 20% for 84% of the analytes. The method presented also a wide linear range (from 9 to 600 μg/kg) and good linearity ($r^2 \geq 0.99$) for the majority of the analytes evaluated. The validated method was applied to the analysis of 51 fruit jam samples of apricot, grape, peach, pineapple and strawberry (from Brazil and from Spain) in order to evaluate the presence of pesticide residues. In total 41 samples (80%) were positive for at least one pesticide and 26 samples (51%) contained at least one pesticide at concentration higher than 10 μg/kg. The most contaminated samples were the strawberry jams with 100% of positive samples and among them, the samples from Brazil were the ones with the largest number of detected pesticides and with the highest concentrations e.g. procimidone at 1,575 μg/kg in one sample. The pesticide more frequently detected was carbendazim, present in 31% of the samples. According to these results, is clearly evident the occurrence of pesticide residues in fruit jams. Thus the control of pesticide residues in these food commodities should be applied because certainly fruit jams contribute for pesticide daily intake of human beings.

[1] N. Touati, M.P. et al., Food Chem. 145 (2014) 23–27.

Keywords: *fruit jams, μLC-ESI-QTRAP-MS/MS, pesticides, QuEChERS*

Acknowledgement: *EURL-FV, CEPARC/UFSM, CAPES*

P7
STABILITY STUDY AND ESTABLISHMENT OF PROCESSING FACTORS FOR PESTICIDES IN THE PREPARATION OF HOME-MADE FRUIT JAMS

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The presence of pesticide residues in raw and processed foods is a consumer concern due to the fact that residues may have negative health effects. The main route for exposure to pesticide residues for consumers is food. Therefore, a proper monitoring and risk assessment of pesticide residues in both raw and processed products is crucial for better consumer protection [1]. The goals of this study were to monitory the stability of 5 pesticides (which have known metabolites) after the preparation of home-made fruit jams from spiked fruits and compare the pesticide residues concentrations in the home-made jams with pesticide concentration in the raw fruits (both spiked at the same level). Thereby a mixture of the pesticides, namely carbendazim, chlorpyrifos, imidacloprid, iprodione and propargite, were spiked to 5 different types of fruits (apple, orange, peach, pear and strawberry) at 500 µg/kg. Jams were prepared with these samples by cooking the milled and spiked fruits with sugar and ultrapure water (ratio, 5:5:2, respectively), in an open pan for 30 min. The QuEChERS extraction method was applied either to blank fruits, to the fruits spiked at 500 µg/kg and to the home-made jams (prepared from spiked fruits at 500 µg/kg). The acetonitrile extracts of the jams were analyzed by high resolution LC–ESI–QTOF–MS/MS to ascertain the occurrence of the metabolites of the 5 spiked pesticides. Moreover, all the samples (blank fruits, spiked fruits and home-made jams) were analyzed by µLC–ESI–QTRAP–MS/MS for the establishment of a processing factor (the pesticide concentration found in the home-made jams/pesticide concentration found in the spiked fruits). None of the five pesticides metabolites were detected in the home-made jams analyzed via LC–ESI–QTOF–MS/MS. By the comparison of the pesticides concentrations found in the jams and in the spiked fruits could be seen a decrease of the pesticide concentrations in the jams. The percentages of the pesticides that remained present in the home-made jams, presented variations depending on the pesticide and the fruit used for the preparation of the jam. But the average percentage for carbendazim, chlorpyrifos, imidacloprid, iprodione and propargite that remained in the jams, was from 50%, 30%, 60%, 50% and 50%, respectively. So, the processing factors of 0.5, 0.3, 0.6, 0.5, 0.5, respectively, could be established. By the absence of the pesticide metabolites in the home-made jams it was possible to conclude that the pesticides remained stable over the jam preparation process. Even when the pesticides concentration decreased from 40–70%, depending on the compound, it was be attributed to a dilution caused by the addition of sugar and water to the spiked fruits to prepare the jams, and because a volatilization of a percentage of the pesticides during the cooking as observed for chlorpyrifos.

[1] L. Martin, et al., Food Addit. Contam. 30 (2013) 466–476.

Keywords: *home-made jams, pesticides, stability study, processing factors, LC–ESI-QTOF–MS/MS*

Acknowledgement: *EURL-FV, CEPARC/UFSM, CAPES*

P8
POLAR PESTICIDE ANALYSIS BY CESI–MS

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Glyphosate is a common broad-spectrum systemic herbicide used widely to kill weeds especially annual broadleaf weeds and grasses known to compete with crops. Usually glyphosate, as it is very polar, undergoes FMOC derivatization by reacting the native glyphosate with Fluorenylmethyloxycarbonyl chloride (FMOC–Cl) before analysis. This derivatization step complicates the analysis and there is a growing need for a method which can detect not only glyphosate but also its major metabolites, for example Glufosinate, in their underivitised state. Standard HPLC methods which have been used to analyze underivitised polar pesticides usually involve HILIC or Hypercarb based separations and are still prone to some technical challenges. In the case of Hypercarb based methods new HPLC columns require conditioning before analysis and other HILC based methods can be prone to the effects of matrix interferences which can cause retention time drifts and analyte suppression. Capillary electrophoresis (CE) is a separation technique designed to separate polar constituents based on their charge and size and is well suited to the separation of small polar contaminants such as Glyphosate. CE runs at extremely low flow rates and although loading amounts are lower than LC based methods this is off set by the huge gains in ion generation and reduction in ion suppression seen at these flows. In this presentation we will show how capillary electrospray ionization (CESI–MS, which has a revolutionary design where the CE flows directly into the MS source at low nL/min) has been used to analyze polar pesticides in food extracts and show how this new technique can reduce the effects of matrix suppression on results and still achieve low limits of detection as well as separation of a selection of underivitised polar pesticides.

Keywords: *CESI, glyphosate, CE–MS, polar pesticides*

P9
A FISH IN THE VINEYARD: TESTING THE EFFECTS OF GRAPE VINE PESTICIDES ON GRAPES, GRAPE JUICE AND WINE USING THE ZEBRAFISH ANIMAL MODEL

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The toxic effects of pesticides residues, as well as of most chemicals, are generally evaluated in laboratory conditions using standard pesticide solutions. This approach is valuable as a starting point and can help understanding the toxic mode of action as well as the general effects produced by the chemicals under study. However, it does not take into account the possible effects of the food matrix and of the possible presence of other chemicals that might interact with the substance modifying its effects. In other words, current toxicology procedure performed in laboratory conditions might lead to an inaccurate and misleading interpretation of toxicity assays. To overcome this problem, in this work we developed a method to test the effects of pesticides residues in real food samples. Specifically, given the wide variety of pesticides used in grape vine pest control, we decided to test the toxic effects of pesticides in grapes, grape juice and wine. As bio logic system for the toxicological assays we used the zebrafish animal model. The first step of our study was to treat real samples to make them suitable to be administrated to the biological system without interfering with its normal living functions and with producing toxicity. Specifically, in the case of grape juice and wine it was first necessary to adjust the pH to values compatible with zebrafish living and well-being (between 6 and 8). Subsequently, samples were filtered through a 0.22 µm filter and extracted with acetone. The extracts were separated into 2 fractions one containing 99%. In both fractions acetone was evaporated and the extracts were diluted. With such preparation protocol, wine samples were only diluted 1:5 and grape juice 1:50 before the exposure to zebrafish embryos. In the case of grapes, they were smashed, extracted with methanol and centrifuged. The supernatant methanol was evaporated and the sample was diluted, whereas the pellet was resuspended in water. After extraction samples were filtered and then diluted in water. Both treated fraction were used for toxicity evaluation. These sample treatment performed on contaminated grapes, juice and wine proved to be suitable for an accurate evaluation of the toxic effects detectable with the zebrafish animal model.

Keywords: *pesticides, toxicity evaluation, real samples, viticulture, zebrafish*

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P10
MODIFIED QUECHERS METHOD COUPLED TO GC QQQ MSMS FOR THE DETERMINATION OF PESTICIDE RESIDUES IN A HERBAL, BOLDO, ITS LEAVES AND THEIR INFUSION AND THE RESULTING TRANSFERENCE TO THE BREW.

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Peumus Boldus (Boldo) is a Chilean medicinal plant consumed worldwide as a tea and known to aid digestion and stomach diseases. Boldo naturally grows in the Chilean forest, but it is also cultivated in America, Africa and Asia. As any agricultural commodity, pre and post harvest pesticides are employed to improve its production. Governments need methods for pesticide surveillance for national consumption and eventually regional export of this product. The current project aims at validating sixty representative GC amenable pesticides in Boldo. This complex analytical matrix contains high amounts of secondary metabolites with similar physicochemical properties to the pesticides under study, interfering with their extraction and detection. Previous studies of our group allowed the selection of a modified citrate buffered QuEChERS method, using different quantities of the cleanup salts for pesticide residue analysis in herbst, for the analysis of 20 pesticides residues using GC–MS. Based on this sample preparation strategy, the method was tested using a GC–QqQ and the validation of pesticides residues in infusion of Boldo leaves was also studied. The methods were fit to purpose with recoveries between 70–120% for the pesticides under study with RSD below 20% and residuals lower than 20% as required by DG-SANCO. These tools were employed to evaluate the transfer of pesticides from the Boldo leaves to the infusion and the processing factor (PF) of each pesticide was estimated. The PF varies according to the physicochemical properties of the pesticides. Pyrethroids showed PF around 10%, fungicides in the range 10–22% but organophsophates showed the greatest variation, from Metidathion (55%) to Ethion (9%).

Keywords: *peumus boldo leaves, GC QqQ MSMS, infusion, pesticides processing factors*

Acknowledgement: *Agilent Tec., FAO IAEA, CSIC UdelaR*

P11
APPLICATION OF Q/ORBITRAP FOR ROUTINE ANALYSIS OF PESTICIDE RESIDUES IN FRUIT AND VEGETABLES

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The very high selectivity of the accurate mass spectrometers makes them a powerful tools in the field of pesticide residues analysis. Modern instruments provide wide possibilities of identification and very good quantitation. In 2005 new type of mass analyser called Orbitrap was introduced into the market. Orbitrap is an electrostatic ion trap. Ions are introduced into the Orbitrap in small packets. A strong electrical field inside the analyser initiates axial oscillation. Ions oscillate harmonically with a period proportional to (*m/z*) 1/2 and produce an image current on split outer electrodes. Subsequently, the signal obtained is converted from time-domain into a mass spectrum by Fourier transformation. In this analyser resolution is inversely proportional to (m/z) 1/2. In other words Orbitrap provides higher resolution for smaller molecules. By that is very convenient for pesticides analysis when the matrix interferences are high. Among the mass spectrometers equipped with Orbitrap are single stage instruments and hybrids with ion trap or quadrupole. In this work quadrupol/Orbitrap hybrid (QExactive) was used. Three resolutions of 17,500, 35,000 and 70,000 (FWHM at *m/z* 200) were compared in various fruit and vegetable matrices with different degree of difficulty. The most important benefits from analysis by applying higher resolution values are: reduction of number of false positive detects reduction of number of false negatives, improvement of peak shape, improvement of peak area reproducibility. MS/MS fragments were used for identification of target pesticides. However MS/MS fragmentation was carried only one time per chromatographic peak. It was sufficient for identification and did not diminish considerably the amount of the data acquired in full scan mode. MS/MS fragments helped to eliminate false positive detects. Finally quantitation by us of Q-Orbitrap was compared with triple quadrupole quantitation. For 93% of the samples the difference between concentration determined by QqQ and LC/Q–Orbitrap MS was lower than 20%.

Keywords: *Orbitrap, QExactive, pesticides, accurate mass spectrometry, high resolution*

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P12
DETERMINATION OF CARBAMATE RESIDUES IN HIGH-FAT CHEESES BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY USING ZIRCONIA-BASED QUECHERS

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Carbamate (CRB) pesticides are used for agricultural activities. The presence of CRB residues in processed foods could have adverse health effects. Due to their high lipid content, cheese may be considered a good indicator of indirect contamination of foodstuffs by CRB residues. The main components of cheese are fat, protein, salt and carbohydrates. Thus, the determination of CRB residues in cheese is still a challenge, as these matrices are highly complex, requiring sample treatments with exhaustive clean-up. In this work a selective and sensitive multiresidue method based on QuEChERS methodology has been evaluated and validated for the determination of 28 CRB residues in in high-fat cheeses using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). The separation of CRBs was achieved in less than 6 min, using a Zorbax Eclipse plus RRHD C18 column (50 mm × 2.1 mm, 1.8 µm), with a mobile phase of water and methanol, both of them with 0.01% formic acid (flow rate 0.5 mL/min, 25°C). The analytes were detected in ESI+ with multiple reactions monitoring mode. QuEChERS methodology has been proposed as a good alternative for extraction and clean-up. Moreover, a new sorbent, SupelTM QuE Z-Sep+ has been tested for dispersive solid phase extraction (dSPE), after extraction of CRBs with acetonitrile. Z-Sep+ was compared with other sorbents previously reported for dSPE of fatty matrices (i.e. mixture of C18 and PSA), resulting in reduced matrix effects (ME) without a significant decrease of analyte recoveries. Thus, ME was studied in different samples (Gorgonzola, Roquefort and Camembert cheeses) being ≤|31|% for all the studied pesticides. Matrix matched calibration were performed with R^2 > 0.991, with dynamic ranges between 5–150 µg/kg. Under optimum conditions, recoveries ranged from 70–115%, with relative standard deviations (RSD) lower than 13%, obtaining limits of quantification within the range of 0.5-5.4 µg/kg, lower than those usually permitted by current European regulations in food matrices. As a conclusion, UHPLC–MS/MS combined with a modified QuEChERS based on the use of Z-Sep+ could be used as a rapid, convenient and high throughput method for clean-up and analysis of pesticide residues in high-fat cheese samples at trace concentrations. The results in terms of sensitivity, selectivity, accuracy, cleanliness of extracts and ME indicated that this new sorbent is an interesting alternative for dSPE of matrices with a high fat content.

Keywords: *carbamates, cheeses, UHPLC–MS, QuEChERS*

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P13
OVERCOMING DIFFICULTIES IN DETERMINATION FOR SELECTED PESTICIDE RESIDUES PRONE TO DEGRADATION, LOW RECOVERIES AND MATRIX INTERFERENCES

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The implementation of multiresidue methods in the laboratory requires recoveries range between 70–120% as a rule. But in some cases these values cannot be achieve and need specific evaluation to overcome the problems affected during the different steps of extraction procedure. This current work seeks to explain the problems observed for some specific pesticides detected very often. During the development of European Union Proficiency Test for Screening Methods in 2015 (EUPT-SM07) it was observed a lack in the detection of diafenthiuron and only 9% of the laboratories reported this compound. Other examples observed were chlorothalonil and ethoxyquin. They presented low recoveries by using QuEChERS method, consequence of the strong matrix interaction. These studies have evaluated the specific condition that have to be applied in the initial extraction step and the necessary pH control to overcome the difficulties committed. In a study carried out with different matrices/extraction methods, interferences with the matrix were found. This phenomenon can lead to errors in identification/quantification, meaning potential false negatives. As example we present the case of azinphos-methyl in onion.

Keywords: *difficulties, low recoveries, matrix interferences, degradation*

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P14
ANALYTICAL VALIDATION AND EVALUATION OF ORGANIC FRUIT AND VEGETABLES AT LOW CONCENTRATION LEVELS BY LIQUID AND GAS CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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In the last report of European food safety authority (EFSA), for the principal food product groups the detection rate and MRL exceedance rate was typically lower for organic products compared to conventionally produced food. However in the case of baby food similar MRL exceedance were found. These data pointed out the interest in to extend the monitoring programs to that specific food group. The present work is focused on evaluating the presence of pesticide residues in organic matrices. The samples were collected from different organic markets and analyzed using liquid and gas chromatography coupled to tandem mass spectrometry (LC–QqQ–MS/MS, GC–QqQ–MS/MS). A total of 306 pesticides and 32 matrices were evaluated using citrate QuEChERS extraction method. LC and GC coupled to tandem mass spectrometry are powerful techniques that provide excellent selectivity and sensitivity, allowing the reliable identification and quantification of the analytes in complex matrices like fruit and vegetables. Taking into account that the maximum residue levels of pesticides or metabolites of pesticides in infant formulae and follow-on formulae range from 0.004–0.008 mg/kg, the current study was validated at 0.005 mg/kg. Using the LC–QqQ–MS/MS method, 30 different pesticide residues were detected in some of the analyzed samples. In a total of 48% of the analyzed samples was detected some of evaluated pesticides and 27% of samples contained pesticide residues at a concentration level higher than 0.010 mg/kg for at least one pesticide residue. In addition, 8% of the samples showed two or more pesticides. In the other hand the results obtained by GC–QqQ–MS/MS were the following, 37 pesticides detected in the evaluated samples. A 53% of positives samples and 18% presented at least one pesticide over 0.010 mg/kg. From the total analyzed samples a 7% reported two or more pesticides. The most frequently detected pesticide residues, independent of the commodity and the employed analytical technique were iprodione, chlorpyrifos, boscalid, bupirimate and ethofenprox. The results obtained in this study have shown that some organic samples contained pesticide residues which are unauthorized under European organic product regulations. The presence of pesticide residues may also be due to cross contamination via water or air, or cultivation on soil previously used for conventional production.

Keywords: *organic, fruit and vegetable, pesticide residues, mass spectrometry*

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P15
EUROPEAN UNION PROFICIENCY TESTS IN FRUITS AND VEGETABLES. MAIN RESULTS OBTAINED DURING THE LAST 10 YEARS

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European Union Proficiency Tests in Fruits and Vegetables (EUPT-FV) date back to 1996, when they were organised for the first time by the National Food Administration in Uppsala (Sweden) in cooperation with the University of Almería (Spain). Since 2004 the European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables (EURL-FV) in the University of Almería, Spain, has organised them on behalf of the European Commission, Health & Consumer Protection Directorate-General (DG-SANCO). The collection of information during the past ten years (EUPT-FV06 to EUPT-FV16) involving European official laboratories for pesticide residue control has generated an important database of more than 20 000 pesticide residue results using Multi-residue Methods (MRMs), and has led to very valuable achievements in areas such as test sample preparation, data dispersion and statistical evaluation; as well as giving an overview as to the effectiveness of proficiency tests as an important tool in the development of quality control results in laboratories involved in food control. Over the years, the number of compounds included in the pesticide target list has increased, as well as the number of participant laboratories. The dispersion of the results submitted by the laboratories has been evaluated with the robust dispersion (Qn RSD), which has decreased from EUPT-FV01 to EUPT-FV17, highlighting the improvement of the participant laboratories. Another achievement in data dispersion is that the big amount of data results has led to a strengthening in the use of the 25 % fit-for-purpose relative standard deviation (FFP-RSD) as well as the use of an internationally accepted 50 % target expanded measurement uncertainty for multiresidue analysis of pesticides.

Keywords: *proficiency tests, fruit and vegetable*

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P16
SCREENING AND QUANTITATION OF ABOUT 200 PESTICIDES IN HONEY BY AN INTEGRATED ON-LINE EXTRACTION UHPLC–MS/MS SYSTEM

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Solid Phase Extraction (SPE) is widely used for sample clean up before LC–MS/MS analysis. Time and money can be saved with integration into the analyzing system. Here we present a simple, cost effective and sensitive procedure for screening and quantitation of pesticides in honey using an integrated On-Line Extraction (OLE) –UHPLC–MS/MS system for analysis of pesticides in honey. A study using the EVOQ LC–TQ analyzed about 200 pesticides in honey using only one method with positive negative switching for 430 MRM transitions. The measurements were conducted by dilute-and-shoot without sample enrichment. The honey was diluted 10-fold and filtered before injection. An YMC-Pack ODS–AQ, 10 µm, 10 mm × 2 mm (I.D.) column was used as trap column. An aqueous mobile phase was used to retain the pesticides on the trap column and to elute the monosaccharides in the honey out to the waste and then the valve switched to couple the trap column with analytical column for separation and detection. The linear range was about 1 to 1,000 ng/g and the linear regression co-efficiency R^2 was >0.99.

Keywords: *pesticides, residues, honey, on-line extraction*

P17
THE USE OF AUTOMATED SHAKING WITH QUECHERS METHODOLOGY TO IMPROVE INCURRED PESTICIDE RESIDUE EXTRACTION EFFICIENCY IN FOOD

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As a result of excellent performance and cost-effectiveness, QuEChERS methodology has become standardized in many labs for pesticide residue analysis. Although most methods were developed and validated using spiked samples, it is necessary to evaluate these methods for extraction of incurred pesticide residues to fully confirm method performance. This work illustrates the use of the QuEChERS procedure to extract existing pesticide residues from a number of produce and/or food samples. Sample shaking was performed either by hand for 1 minute, as instructed by the QuEChERS methods, or by using a simple shaker/vortexer for 10 minutes. Extraction efficiency for both procedures was compared. For the tested food samples, the yield of the incurred pesticides using the shaker protocol was observed to be 10–15% higher than the yield resulting from hand shaking. It was concluded that the use of this inexpensive shaker to the laboratory workflow can significantly impro ve measurement accuracy as well as pesticide yields.

Keywords: *pesticides, food contamination, sample preparation, automation, GC–MS*

P18
USE OF GRAPHITIZED CARBON BLACK AND ZIRCONIA-BASED ADSORBENTS FOR THE REMOVAL OF PIGMENTS DURING QUECHERS

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The clean-up of fruit and vegetable samples for pesticide analysis is complicated by the presence of plant pigments in the matrix. Pigment molecules are generally large, non-volatile compounds that tend to get trapped in the inlet liner of the GC–MS. Use of the QuEChERS clean-up method does not necessarily remove all pigments from the matrix. Method EN 15662 recommends the addition of Graphitized Carbon Black (GCB) to remove plant pigments from samples. In addition to removing plant pigments, GCBs also retain some pesticides, especially planar pesticides. A range of plant pigments was chosen to be representative of the pigments found in various fruits and vegetables. In addition to GCB, the zirconia-containing adsorbents Z-Sep and Z-Sep+ were used to remove the pigments from acetonitrile solutions designed to simulate dispersive SPE extracts. As expected, the high surface area SupelcleanTM ENVI Carb removed the plant pigments very well with the excepti on of the highly water soluble betanin. SupelTM Sphere displayed a similar pattern with the exception of crocin. Carbons with lower surface areas retained less plant pigment material. ZSep+ removed between 40% and 80% of the plant pigment from the solutions. The addition of zirconia to the surface of the carbon appears to increase pigment retention.

Keywords: *pesticides, food contamination, sample preparation, QuEChERS, GC–MS*

P19
MULTI PESTICIDES RESIDUE DETERMINATION IN FRESH OKRA USING QUECHERS SAMPLE PREPARATION AND GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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A fast and sensitive method has been developed and validated for the analysis of 90 pesticides in fresh okra using the QuEChERS sample preparation technique. Sample preparation was optimized in order to eliminate chlorophyll pigment and other co-extractives which can cause retention time shifting, affect chromatographic peak shape and cause loss of sensitivity of the target analytes. The graphitized carbon black (GCB) which adsorbs colored pigments was tried with other sorbents including C18 (alkylated silica) and primary secondary amine (PSA) for the effective cleanup of the acetonitrile extracts. The optimized sample preparation method involved extraction of 2 g of sample with 10 mL of water and 10 mL of acidified acetonitrile in the presence of sodium acetate buffer (1.5 g) and magnesium sulphate (6 g) (Agilent P/N5982-5755). Cleanup of the acetonitrile extract was achieved using 50 mg PSA and 50 mg C18, 7.5 mg GCB and 150 mg of magnesium sulphate (Agilen t P/N 5982-0028). The limit of quantification (LOQ) for most of the compounds was 0.99 within the calibration range of 0.3–200 ng/mL. The quantification of the residues was performed using matrix matched standards prepared in residue free organic red chili powder. The recovery at 10, 25 and 50 ng/mL was within 70–120% (n=6) with associated relative standard deviations below 20%, indicating satisfactory intra-laboratory precision. Chromatographic and mass spectrometric parameters were set up using the Agilent Pesticide and Environmental Contaminants Database (G9250AA). Target analyte MS/MS transitions were selected in order to give the best selectivity against the matrix. Cold splitless injection was performed with a retention time locked (RTL) method. Carryover of high volatile matrix compounds in subsequent runs was eliminated using capillary flow technology with mid-column back flus hing. The method was applied for the analysis of 25 samples and it was found that the method was suitable for the routine analysis of the 90 target compounds evaluated in this study.

Keywords: *GC–MS/MS, GC/MS, okra pesticides, QuEChERS, retention time locking*

P20
FAST SCREENING, IDENTIFICATION AND QUANTIFICATION OF PESTICIDE RESIDUES IN BABY FOOD USING GC ORBITRAP MS TECHNOLOGY

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Pesticides are a diverse group of substances widely used to control plant pests in order to improve crop production. Current trends indicate that > 500 compounds are routinely used globally, with different countries having varying regulations concerning licensing and maximum residue levels (MRLs). With increasing global trade there is a requirement for multi-analyte screening strategies capable of efficiently and confidently detecting both targeted and unexpected residues in food commodities to ensure consumer safety. Recent advances in MS technology has shown the potential for the analyses of complex samples. This study shows the performance of a rapid GC method in combination with a novel benchtop GC Orbitrap mass spectrometer to rapidly screen, identify and quantify pesticide residues in baby food. Thermo Scientific Q Exactive GC system was used to analyse 132 pesticides in standards and QuECheRS acetonitrile extracts of baby food. A fast GC-MS method (11 minutes) was developed using a TG-5SilMS (15 m × 0.25 mm × 0.25 µm) GC capillary column. Data was acquired in full scan at 60,000 resolution (FWHM at *m/z* 200). Calibration curves ranging from 0.5–100 ng/g (or 1.0–200 ng/g) were acquired for target quantitation of samples. The identification of pesticides were based on this criteria: retention time match within 0.1 minutes of a standard. Measured accurate mass of the diagnostic fragment ions within

Keywords: *pesticides, GC Orbitrap, mass spectrometry, baby food, accurate mass*

P21
DEVELOPMENT OF AN ACCELERATED SOLVENT EXTRACTION (ASE) METHOD WITH IN-LINE CLEAN UP FOR THE EXTRACTION OF PESTICIDES FROM FISH MEAL AND FISH OIL MATRIX

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Accelerated solvent extraction (ASE) provides a combination of increased temperature and pressure to improve the efficiency of sample extraction. This poster describes the application of ASE as a possibility to combine extraction and sample clean up into one single step. The method development was performed using the extraction of representative pesticides from fishmeal and fish oil. The main challenge was to accomplish a compromise between efficient clean up and recovery of the analytes. First step of the method development was the monitoring of the clean up efficiency. The obtained extract should be colorless and fat free which was verified gravimetrically. Different fat retainer materials (florisil, silica gel, sulfuric acid impregnated silica gel, acidic alumina) were included into the method development. Besides finding the best retaining material for the requested application, the ratio of sample and fat retainer was an important point to be considered. This ratio depends on the composition of the extraction solvent. To find the best conditions, different solvents ranging from hexane to more polar solvent mixtures were tested. Additionally, the extraction temperature, extraction time and the number cycles during the extraction were optimized. The extraction conditions that showed a sufficient clean up were used to extract spiked fish meal and fish oil samples followed by GC–MS/MS analysis to determine the recovery.

Keywords: *accelerated solvent extraction, pesticides, PCB, in-line clean up*

P22
COMPLETE PESTICIDES WORKFLOW – COMBINATION OF LC-MS/MS AND LC–HRMS ANALYSIS

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Pesticide residues are still one of the most challenging contaminant groups routinely analysed in most of the laboratories controlling food and food products. The challenges are the huge number of existing and possibly applicable (>1100) pesticides on one hand and their chemical diversity on the other hand. To be able to effectively control the wide range of matrices and target pesticides, reliable and validated multi methods are required using the latest technical developments. The aim of our presentation is to report on a complete solution package for pesticides workflow comparing two analytical top-end techniques: liquid chromatography-triple quadrupole mass spectrometry and liquid chromatography-high resolution accurate mass spectrometry. Both methods were validated according to the European SANCO guidelines 12495/2011. Analytical parameters such as linearity, specificity, LOD, LOQ, precision and accuracy were evaluated. For validation fortified homogenized samples and commercially available certified reference materials were used. Three matrices were chosen for the validation study: strawberry representing fruit with high acid content, leek representing vegetable with high pigment content and wheat flour representing matrix with low water content. Tea and honey as more complex and complicated matrices were used for the high-resolution accurate mass spectrometry (HRAM–MS) measurements. The QuEChERS extraction procedure was applied on all investigated matrices. The validation outcome showed satisfactory results for both methods since LOQs ≤ 10 µg/kg were reached for the majority of the 320 target compounds in all matrices. The recoveries were typically in the range from 70–120% for most of the compounds (>270) determined at two levels with slightly worse results for leek and flour comparing to strawberries. The RSD values for most of the compounds (>270) were lower than 20%. HRAM–MS data showed very comparable results to the triple quadrupole based method. This poster will discuss the data in detail.

Keywords: *pesticides multi method, LC–MS/MS, LC–HRMS*

P23
DETERMINATION OF HIGHLY POLAR NON-QUECHERS-AMENABLE PESTICIDES IN FOOD OF PLANT ORIGIN

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In 2006 the EU Reference Laboratory for Single Residue Methods (EURL-SRM) started a method development for the determination of a group of highly polar non-QuEChERS-amenable pesticides. This simple method consists of an extraction step with acidified methanol, if necessary after water addition and a centrifugation step followed by filtration. The analysis is finalised by LC-MS/MS determination. Since then, the method was updated continuously. The current version 8.1 of the so-called QuPPe (Quick Polar Pesticides) method covers nearly 40 pesticides, 27 of which can be determined with only two LC-methods (method M1.3 "Glyphosate & Co. Hypercarb" and method M4.1 "Quats & Co. Obelisc R") [1] The German NRL for Pesticides - section SRM - validated both of these methods including a modification concerning an additional freezing-out for matrices with high fat, starch or protein content. By carrying out this additional clean-up, the interferences of matrix ions and system maintenance could be reduced, thus also allowing a reduction of the detection and quantitation limits.
This poster shows the validation data for method M1.3 including glyphosate, ethephon, glufosinate, fosetyl-Al, maleic hydrazide and their metabolites as well as perchlorate and chlorate. The validation covers five matrices (cucumber, wholemeal rye flour, lemons, raisins and avocado) at three different levels (0.01, 0.05, 0.10 mg/kg) each. The validation was carried out according to the AQC-document [2] for a quantitative method with recoveries between 70 and 120%, and within-laboratory reproducibility RSD_{wR} of $\leq$ 20 %. With only few exceptions QuPPe method M1.3 "Glyphosate & Co. Hypercarb" could be validated on all levels for all analyte-matrix-combinations. Most difficulties were observed concerning phosphonic acid and for few analytes to be determined in wholemeal rye flour, lemons and raisins. Additionally, data sets of matrix-matched calibration and solvent calibration are also shown.

[1] quppe.com
[2] Guidance document on analytical quality control and validation procedures for pesticide residue analysis in food and feed. SANCO/12571/2013

Keywords: *QuPPe method, Glyphosate & Co., matrix matched calibration, solvent calibration*

P24
MULTI-RESIDUE PESTICIDE ANALYSIS OF FOOD SAMPLES USING ACETONITRILE EXTRACTION AND TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

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Two dimensional liquid chromatography coupled with tandem mass spectrometry operated in dMRM mode (2D–LC–MS/MS) seems to be perspective method of choice for targeted multi-residue analysis of complex food matrix samples. In recent years a fully automated EPICS® (Easy Pesticides Isolation and Concentration System) 2D-LC-MS/MS system allowing removal of co-extracted matrix compounds and subsequent separation of pesticides on both reversed phase and HILIC columns in single chromatographic run has been introduced to the market. It provides possibility to inject raw acetonitrile extract and analyse pesticide residues from different commodities. The benefit of 2D-LC is that highly polar compounds are very well separated on HILIC column and at the same time co-extracted compounds from matrix are caught on HILIC. Therefore, HILIC column may replace clean-up steps used in conventional methods or in QuECHERS extraction. Experimental set-up used in presented method allows to improve (i) peak shape of polar compounds and (ii) separation of analytes from the matrix compounds. Presented method covers a wide polarity range of pesticides including organochlorines, organophosphorous, pyrethrins, pyrethroids, carbamates, benzimidazoles, triazines, substituted ureas and organotin compounds. Basic validation parameters including repeatability, recovery, linearity, LODs, LOQs for more than 200 pesticides in plant matrices is presented. Within-laboratory reproducibility of presented method is evaluated as well.

Keywords: *2D-LC–MS/MS, pesticides, fruit, vegetables, foods*

P25
COMPARISON OF HIGH-FLOW AND MICRO-FLOW UHPLC/MSMS (INCORPORATING POLARITY-SWITCHING) FOR THE QUANTITATIVE DETERMINATION OF MULTIPLE PESTICIDE RESIDUES IN CRUDE FRUIT AND VEGETABLE EXTRACTS

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Matrix effects in pesticide residue analysis are a constant concern for laboratories engaged in monitoring schemes. Various sample preparation and clean-up strategies can help to minimise or ideally eliminate the effects of matrix. Laboratories that prefer to analyse crude extracts directly by LC/MSMS are attracted to complementary instrument technologies that could improve this analysis of 'dirty' samples. Sample introduction via micro-flow LC should be beneficial for the analysis of crude extract in particular because there is a significant reduction in the amount of solvent and matrix entering the MS ionisation region compared to general UHPLC flow rates. In addition, low micro-flow rates can result in an increased sensitivity since electrospray ionisation processes can become more efficient by reducing the magnitude of the 'spray' and initial droplet size. There is also an obvious environmental and economic advantage of using micro-flow LC routinely due to the dramatic reduction in solvent consumption compared to high flow LC. In our evaluation, we decided to compare high-flow (400 µl/min) and micro-flow (25 µl/min) LC/MSMS experiments via the analysis of crude extracts from various fruit and vegetable samples received as part of the UK Pesticide Residues in Food annual surveillance programme. In total, 192 pesticides and their metabolites were sought using a single Multiple Reaction Monitoring (MRM) experiment incorporating polarity ESI switching and scheduled MRM i.e. 364 MRMs in positive mode and 20 MRMs in negative mode. This poster presents a comparison and assessment of results obtained from micro-flow and high-flow (UHPLC) MSMS experiments conducted on solvent and matrix-matched standards as well as retail samples. Data are presented from a small subset of analytes.

Keywords: *pesticide, LC/MSMS, micro-flow LC, fruit, vegetable*

P26
PESTICIDES TARGET SCREENING WITH AN ATMOSPHERIC PRESSURE CHEMICAL IONISATION GC COUPLED TO HIGH-RESOLUTION QTOF–MS

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The use of accurate mass QTOF–LC/MS with electrospray ionization for target pesticide screening enables the identification of hundreds of pesticides in single runs. On the other hand GC/MS is well covering these compounds, generally exhibiting lower chemical background and less matrix effects. In this study, we combine the advantages of GC and QTOF technologies coupling a GC–APCI interface to a QTOF mass spectrometer. Analyses were performed using a 60 Pesticide Standard Mix which we had introduced earlier [1]. The pesticides were selected according to their relevance in today´s routine food analysis and according to their chemical characteristics as molecular mass, chemical composition, their polarity and volatility. The mix contains amongst others: Azinphos-Methyl, Chlorpropham, Diazinon, EPN, Imazalil, Indoxacarb and Myclobutanil. Standards were spiked into fruit and vegetable matrices. Alternating measurements with full scan and broadband CID (bbCID) enable identification and verification even of compounds small in abundance. GC peaks typically show FWHM between 1-3 seconds. With 8 Hz data acquisition we obtained excellent coverage of these sharp peaks resulting in reliable peak areas for quantitation. Limits of quantitation (LOQ) were for 64% of analytes below ≤ 10 pg/µL, limits of detection (LOD) were for 92% of analytes below ≤ 10 pg/µL. Mass accuracy of the calibrant runs only was below 0.8 ppm averaged over all calibrant runs and all analytes. Average mass accuracy raised slightly to 0.9 ppm when all matrix sample runs were included into mass accuracy calculation. In summary we observed only small influence of matrix samples onto mass accuracy. Here we developed a base basic method using GC–APCI–QTOF which in future should be developed further towards a more comprehensive GC–APCI–QTOF method covering hundreds of pesticides.

[1] P. Decker et al. RAFA Conference 2011, Poster O-25, O-27

Keywords: *GC–APCI, QTOF–MS, pesticide, screening*

P27
A SIMPLIFIED LC-MS/MS METHOD FOR DETECTION, IDENTIFICATION AND QUANTIFICATION OF OVER 400 PESTICIDES IN COMPLEX MATRIX WITHOUT COMPROMISING DATA QUALITY

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More than 900 compounds are routinely used as active ingredients for crop protection across the globe with many more legacy compounds now banned. With increasing global trade there is a requirement for rapid multi-residue screening and confirmation methods to efficiently determine residue violations and protect consumers. Effective multi-residue methods rely on management of the acquisition of a large number of MRM transitions. Setting up overlapping MRM windows based around the retention time of each analyte ensures that no time is wasted acquiring other transitions for compounds that have yet to elute. This optimises the time spent acquiring data to maximize sensitivity whilst ensuring sufficient number of data points across peaks to give good precision. One disadvantage of retention-time window based MRM acquisition is the need to make regular checks on retention time drift and to make any necessary adjustment of the acquisition method before analysis. In addition, retention time must be stable throughout multiple analyses regardless of changes in commodity/matrix being analysed. Modern source transfer optics and collision cell technology minimise source-to-detector ion transit times for high-speed data acquisition. Here we demonstrate the use of rapid cycle times to enable the use of relatively wide acquisition windows, without any loss in performance, which removed the need for regular adjustments to the acquisition method before each analysis and has eliminated the impact of matrix on retention time drift. A single, fast method for detection, identification and quantification of more than 400 pesticides has been developed. All pesticides were chromatographed on a reverse phased UPLC column within 12 minutes. Two MRM transitions for each pesticide were monitored using both electrospray ionisation, deploying polarity switching. Data showing the performance of the method will be presented, including the quantitative analysis of pesticides at very low concentrations in chilli powder and assessment of robustness from a large number of injections.

Keywords: *pesticides, multi-residue methods, UPLC–MS/MS, acquisition, ease of use*

P28
COMPUTATIONAL AND BIOSENSOR INVESTIGATION OF MOLECULAR IMPRINTED POLYMERS FOR SELECTIVE EXTRACTION OF PHOSMET FROM OLIVE OIL

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The most extensively applied agrochemicals in olives cultivation are organophosphorus insecticides. They provide accurate, rapid and cost-effective treatment but become dangerous if they persist at the harvest stage. If the contaminated olives are used in further food production they may present a high risk for human health. Additionally, the majority of these pesticides are lipophilic and are able to remain in oil for extended periods of time. To reduce potential risk of high level of pesticides in the products, the European Union and the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO) have determined maximum residue limits which are acceptable and not harmful to humans (MRLs). Following these restrictions it became very challenging to find a suitable method for quick detection and monitoring of minimal concentration of pesticides in olive oil, as the samples are highly complex and mainly contain triglyceri des (98-99%). This work presents the development of molecularly imprinted polymers (MIPs) for the selective extraction of phosmet from olive oil. Phosmet is an organophosphorus insecticide (OPs) that is used in large quantities in olive plantations. As all OPs, phosmet residues can survive to the harvest stage, so that trace amounts of these pesticides are likely to be found in olive oil. Computational simulations allowed selecting N,N'-methylenebisacrylamide (MBAA) as the monomer showing the highest affinity towards phosmet. Experimental validation of this selection was realized using highly sensitive biosensors based on genetically modified acetylcholinesterase from Drosophila melanogaster. The target was incubated with the selected monomer in several solvents, 4 µL of each mixture was injected in biosensor cells containing 8 mL of phosphate buffer in presence of N-bromosuccinimide, and inhibition measurements were recorded. The results showed a best affinity between phosmet and the selected monomer in dimethyl sulfoxide (DMSO), as the inhibition effect of phosmet was shown to decrease while increasing the incubation time with MBAA. New MIPs were fabricated and used for phosmet extraction in solid phase extraction columns (MISPE), which were fed using a syringe injection pump. The developed MIPSE was finally investigated for the selective extraction of phosmet from spiked samples of olive oil.

Keywords: *phosmet, molecularly imprinted polymer, biosensor, acetylcholinesterase, olive oil*

P29
SCREENING FOR PESTICIDES RESIDUES IN EGGS APPLYING LC–Q–TOF MS AND LC–Q–ORBITRAP MS

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Screening with modern accurate mass instruments such as LC–Q–ToFMS or LC–Q–OrbitrapMS gives the analyst the possibility to quickly obtain the residues profile of samples. These systems provide high resolution mass spectra as well as classical MS/MS experiments as in triple quadrupole (QQQ) instruments. The most important difference to classical LC–MS/MS (QQQ) is that the number of analytes per analysis is not limited, at least in principle. Pesticide residues can occur in feed of laying hens and, as a consequence, in eggs. Therefore, for a wide range of pesticides MRLs have been set for eggs. An effective broad scope screening method is required to check for the presence of pesticides at low concentrations. A large number of pesticides can occur in eggs samples, although besides some dual use analytes (substances used as pesticides and as veterinary drugs) only a few pesticides will be expected to be found in most samples, usually on a low level. The EURL AO has developed a method for pesticide residues screening in eggs with LC–Q–ToF MS. Samples were quantitatively extracted and cleanup using an established modified QuEChERS method. The aim of this work was to assess if this screening method is able to meet the requirements of Regulation (EC) No. 396/2005. Therefore, internal databases with chromatographic and spectral data of pesticides were established for more than 250 pesticides. The validation procedure was performed according to the document SANCO/12571/2013. Therefore the extracts were analyzed using both, a LC–Q–ToF MS system and a LC–Q–Orbitrap MS system. The results of the validation of this method will be presented. The results achieved by the two systems do not show substantial differences. Most of the differences can be attributed to different chromatographic conditions used. Comparing the MRL values and the screening detection limits achieved, it is possible to identify a large majority of pesticides spiked into eggs at or below the respective MRL-level. Therefore screening with LC–Q–ToF MS or LC–Q–Orbitrap MS are useful tools to improve the scope of pesticide analysis. Further details of the results will be presented in the poster.

[1] Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method; German version EN 15662:2008 (Foreign Standard)
[2] REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC
[3] SANCO/12751/2013. Method validation and quality control procedures for pesticides residues analysis in food and feed. http://ec.europa.eu/food/plant/protection/pesticides/docs/qual control_en.pdf.

Keywords: *pesticides, screening, honey, ToF, Orbitrap*

P30
THIABENDAZOLE ANALYSIS IN GRAPE AND WINE SAMPLES BY ELISA AND AN INTEGRATED ON SILICON INTERFEROMETER SENSOR

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Benzimidazole fungicides, such as thiabendazole, are pesticides widely used on a broad range of crops as systemic fungicides to control pre-harvest diseases and post-harvest spoilage during storage. As a consequence, residues of these substances can be found in food commodities implying a potential risk for human health. During the last years, novel antibody-based kits for pesticide determination have been introduced into the market with different applications. It has been proved that ELISAs show specific response against a particular substance and are capable of rapid and simple detection of trace of chemical hazards in complex matrices. In this work, we aim to develop and validate a new pesticide residues direct competitive ELISAs for quantitative detection of thiabendazole in grape and wine samples. The results were also compared with those received by integrated on silicon chips Mach-Zehnder interferometric immunosensor arrays. For sample preparation 15 g of sample was mixed with 3 mL of acetonitrile and 12 mL of milliQ water, centrifugued for 5 min, diluted with solvent for LC–MS/MS or diluted with de assay buffer for the ELISA analysis and analysed by injecting 10 µl into the system or by following manufacturers´ protocol for ELISA. The analysis in the sensor was performed for wine sample after 30-times dilution with assay buffer while for the grape samples, the homogenate was first centrifuged and then diluted 100-times with assay buffer. Chips were coated with a thiabendazole-bovine serum conjugate and blocked, prior to the fluidic module application. For the assay, mixtures of calibrators or diluted samples with the mouse monoclonal anti-thiabendazole antibody are run over the sensor for 5 min. The assay in matrix had a detection limits ranging from 0.2–0.6 ng/mL, IC50 of 6–10 ng/mL, dynamic range 0.6–83 ng/mL.

Keywords: *pesticide residue, ELISA, sensor, LC–QqQ–MS/MS, thiabendazole*

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P31
ANALYSIS OF MULTI-PESTICIDE RESIDUES IN SOYBEAN PRODUCTS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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In recent years, multi-pesticide residues analysis method by GC–MS/MS and LC–MS/MS are commonly used for analyzing different types of pesticides and their residue levels in various agricultural products. The present study was aimed to analyze the occurrence of pesticides residue levels in soybean products using the QuEChERS method by liquid chromatography tandem mass spectrometry (LC–MS/MS). The QuEChERS method by MS/MS detection provides good methods of identifying and quantifying numerous pesticides in food products. In the current study, we compared different preparation procedures based on the QuEChERS method for the analysis of 252 pesticides in processed soybean products by LC–MS/MS. For this study, four different types of sample preparation procedures were performed namely non-preparation (Control); 25 mg PSA, 150 mg $MgSO_4$ (Treatment-1); 25 mg PSA, 150 mg $MgSO_4$, 25 mg C18 (Treatment-2) and 25 mg PSA, 150 mg $MgSO_4$, 25 mg GCB (Treatment-3) as purificatio n. The recovery and reproducibility were evaluated by spiking pesticides standards in soybean products at 10 and 50 µg/kg. The analysis was performed five times at each level and the matrix-matched standard calibration was used for quantitative analysis. In soybean paste 239, 212, 220, 224 pesticides and 5, 17, 14, 10 pesticides at C, T1, T2 and T3 were showed in the range of 70~120% and 50~70% recovery with CV ≤ 20%, respectively. In soybean sauce 234, 232, 226, 178 pesticides and 5, 12, 17, 19 pesticides at C, T1, T2 and T3 were found in the range of 70~120% and 50~70% recovery with CV ≤ 20%, respectively. The recovery of ethoxysulfuron, imazosulfuron, chlorsulfuron, cyromazin, gibberellic acid was about 50% in all methods. The results showed that the QuEChERS sample preparation with non-purification or purification with absorbent can be applied for the multi-residue analysis of pesticides in soybean products.

Keywords: *soybean products, LC–MS/MS, pesticide residues, sample preparation*

P32
MODERN STATE OF GC-MS/MS PESTICIDE ANALYSIS USING THERMO SCIENTIFIC™ TSQ™ 8000 EVO AND CHROMELEON™ 7.2 CDS

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Triple quadrupole mass spectrometers such as the Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS systems have gained popularity over their single quadrupole counterparts because of their high selectivity and lower detection limits, especially in complex matrices such as those encountered in pesticide analysis in food. In this poster we present results of GC-MS/MS analysis of pesticides using timed-Selective Reaction Monitoring (t-SRM).The t-SRM optimized dwell times combined with the Enhanced Velocity Optics (EvoCell collision cell) present in the TSQ 8000 Evo enables us to monitor multiple confirming transitions per analyte for a more confident confirmation without compromising quantitation sensitivity. The results we show were obtained using Thermo Scientific™ Dionex™ Chromeleon™ 7.2 CDS software, which combines powerful data analysis capability with easy pesticide analysis method creation. The Chromeleon software pesticide analyzer database contains retention times and transitions for over 1000 pesticides and other compounds of environmental interest. Historically, developing MS/MS transitions for compounds used to be arduous and time-consuming process prone to operator error, but this no longer is the case. This poster highlights the power of AutoSRM which is a tool for developing and optimizing transitions for compounds that are not yet present in the database with simple user interaction and high degree of confidence in the results.

Keywords: *pesticides, Chromeleon™ 7.2 CDS, TSQ™ 8000 Evo GC–MS/MS, timed-SRM*

P33
EVOLUTION OF PESTICIDES IN WINE GRAPE TREATED IN FIELD DURING THE MATURING PROCESS

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The use of pesticides in vineyards of the DOCa Rioja has increased the last years. Vivando® (50% metrafenone) and Collis® (20% boscalid, 10% kresosim-methyl) have been used to control powdery mildew in grapes vines. Vivando® is a pesticide whose active substance is the metrafenone, a benzophenone fungicide with protectant and curative properties intended for the control of this disease. The active materials of Collis® are the boscalid, a carboxamide with protectant, foliar absorption, inhibits spore germination and germ tude elongation effects, and kresoxim-methyl, a strobilurin with protective and curative effects against a wide range of fungal diseases. The maximum residues levels (MRLs) in wine grapes are 5.0, 1.0 and 5.0 $mg \cdot Kg^{-1}$ for metrafenone, boscalid, kresoxim-methyl respectively. It has been studied the evolution in the field of the pesticides in grapes from the application of these to the harvest. In this study, the pesticides have been extracted using a modification of QuEChERS [1] (quick, easy, cheap, effective, rugged, and safe) using dicloromethane as solvent instead of acetonitrile. The ultra performance liquid chromatography combined with time-of-flight mass spectrometry (UPLC–MS) and gas chromatography (GC–MS) are methods used for confirmation and quantification of these pesticides. The concentrations of these pesticides in the harvest are lower than MRLs.

[1] M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F.J. Schenck, JAOAC Int 86(2) 412–31 (2003)

Keywords: *pesticides residues, wine grapes, metrafenone, boscalid, kresoxim-methyl, UPLC-MS, GC-MS*

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P34
PESTICIDE RESIDUES IN FRUIT JUICES PRODUCED IN SERBIA

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Liquid chromatography – tandem mass spectrometry with electrospray ionization (LC–MS/MS–ESI) was applied for simultaneous determination of residues of 49 pesticides in fruit juices after extraction and purification using the QuEChERS method EN 15662. The LC analysis was carried out using an Agilent 1200 HPLC system equiped with a reverse-phase C18 analytical column Agilent Zorbax Eclipse 50×4.6 mm with 1.8μm particle size. The mobile phase consisted of methanol and water with 0.1% formic acid in gradient mode, with the flow rate of 0.6 ml/min. For the mass spectrometric analysis, an Agilent 6410B Triple-Quad LC/MS system was used. Laboratory method performance was characterized with limits of detection and quantification of 0.0005 and 0.01 mg/kg, respectively, recoveries in the required range (70–120%), precision in terms of relative standard deviation <20%, linearity range from 5.0 to 250.0 ng/ml. In total 112 samples, all produced in Serbia, were collected in original packages from the supermarkets in the city of Novi Sad in 2014. Samples included 83 fruit juices with a volume size from 0.75 to 2 L and 29 fruit juices in 0.2 L pack with straw. Pesticide rezidues were found in 76% of juices and 79% of juices with straw. Out of 49 investigated pesticide residues, 15 were detected in at least one sample of juices and 13 in juices with straw. Most frequently found pesticides were carbendazim (51% of juices and 55% of juices with straw), acetamiprid (34% and 52%) and pyrimethanil (17% and 38%). Frequency of detection of imazalil, flutriafol and spiroxamine in juices was 7–8% and azoxystrobin in 14% of juices with straw. The highest concentrations (mg/kg) of carbendazim in juices and juices with straw were 0.023 and 0.010, respectively; acetamiprid 0.011 and 0.007, and pyrimethanil 0.027 and 0.011. Regarding positive samples, the mean concentration of carbendazim was around 0.005mg/kg and acetamiprid 0.003 mg/kg in both groups of samples, whereas pyrimethanil mean ranged from 0.004 in juices with straw to 0.009 mg/kg in juices. The highest recorded concentrations regarding all investigated pesticides were 0.629 mg/kg of spiroxamine in juices and 0.013 mg/kg of endosulfan alpha in juices with straw. With regard to multiple residues, 18% of juices and 38% of juices with straw showed presence of three or more pesticide residues (maximum five and six, respectively). Generally, this investigation revealed high incidence but very low level of pesticide residues in fruit juices produced and marketed in Serbia.

Keywords: *pesticide residues, fruit juice, LC–MS/MS*

P35
MULTI-COMPOUND AND MULTI-CLASS IDENTIFICATION AND QUANTIFICATION USING HIGH RESOLUTION LC–MS/MS

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LC–MS/MS is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues and contaminants. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers limits the number of compound to quantify and identify. In addition there is an increasing demand for retrospective and possibly non-target data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC–MS/MS run. Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were diluted to minimize potential matrix effects and subsequently analyzed by LC–MS/MS using a SCIEX QTOF system operated in high resolution accurate mass MS and MS/MS mode. Full scan MS and MS/MS data was explored to identify targeted compounds using extensive XIC lists of target compounds. Analytes were identified with high confidence based on retention time matching, mass accuracy, isotopic pattern, and MS/MS library searching. Quantitation was achieved in the same data processing step. The developed method was successfully applied to the analysis of store-bought samples. It was found that the use of MS/MS information is crucial to minimize false positive results.

Keywords: *pesticides, LC–MS/MS, high resolution, TOF, quantitation*

P36
IDENTIFICATION, QUANTITATION AND CONFIRMATION OF PESTICIDES IN FOOD SAMPLES USING LC–MS/MS AND ULTRA-FAST POLARITY SWITCHING

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Recent regulations on food analysis require the screening for pesticides using confirmatory techniques, such as GC–MS(/MS) and LC–MS/MS. With more than 1000 pesticides of more than 100 compound classes there is a demand for powerful and rapid analytical methods, which can detect very low concentrations in food matrices. Here we present a high sensitivity and high selectivity LC–MS/MS method that combines Multiple Reaction Monitoring (MRM) quantitation with MRM ratios as a first step of identification. Samples were re-analyzed using LC–MS/MS using information dependent acquisition (IDA) of QTRAP® MS/MS spectra. MS/MS spectra were searched against extensive mass spectral libraries for high confidence confirmation. Food samples, including a variety of fruits and vegetables were extracted using a QuEChERS procedure and injected into LC-MS/MS after extensive dilution to minimize or possibly eliminate matrix effects. LC separation was performed using a Phenomenex core-shell Kinetex Biphenyl column and a gradient of water and methanol and ammonium formate buffer with a total run time of 15 min. Detection was performed on the SCIEX QTRAP® 6500+ system using Electrospray Ionization (ESI). First injection was performed using the Scheduled MRM™ pro algorithm and ultra-fast polarity switching (5 msec) to reproducibly and accurately monitor approximately 600 transitions for the quantitation and identification of 300 pesticides. In a second injection already identified pesticides were confirmed based on MRM-IDA-MS/MS analysis. The acquisition of full scan MS/MS spectra helped to reduce false positive findings. Data processing was performed using MultiQuant and MasterView software.

Keywords: *pesticides, LC–MS/MS, matrix effects, quantitation, identification*

P37
HIGH SENSITIVITY AND SELECTIVITY USING MICROFLUIDIC DEVICE WITH COLLISION CROSS SECTION ION MOBILITY FOR SCREENING ANALYSIS OF PESTICIDE RESIDUES IN FOOD

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Pesticide residue analysis in food has become more challenging considering the increasing number of compounds/complex food commodities to be monitored at low concentrations with generic extraction procedures. The direct consequences are complex extracts with the presence of matrix compounds that can impact the ionisation efficiency of the targeted compounds. To circumvent this matrix effect, diluting the extract was proven to be very efficient but is mostly limited by the sensitivity of the instrument. In some cases, detection can still be made but due to the dilution process other confirmation criteria are lacking for the identification of the compound (e.g. presence of fragments or characteristic isotopic pattern). Hence, data quality is of profound importance to insure correct identification. The study undertaken aims to illustrate how sensitivity enhancements can be achieved using a microfluidic chromatography device and hyphenated to an ion mobility high resolution mass spectrometer, higher selectivity is added to the overall analysis method. Ion mobility mass spectrometry (IM–MS) offers some unique advantages to profiling complex mixtures. We will demonstrate the large gain in signal-to-noise for pesticides in different matrix extracts and how ion mobility enables identification where only the mono-isotopic peak is detected. For example, pencycuron was detected at concentration as low as 200 fg on column but the identification was enabled only by the use of collision cross section (derived from ion mobility separation) as an additional identification point.

Keywords: *ion mobility, signal-to-noise, pesticides, microfluidic chromatography*

P38
MULTI-RESIDUE DETERMINATION OF PESTICIDES IN DAIRY PRODUCTS BY TRIPLE QUADRUPOLE GC–MS/MS TECHNIQUE

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The Italian National Reference Laboratory (NRL) for Pesticide Residues in Food of Animal Origin and Commodities with High Fat Content has adopted a multiresidue method for the determination of pesticide residues in food of animal origin. This method has already been validated for matrices with fat content up to 10% as baby foods of animal origin [1], egg and fish [2]. The same method has been tested for 90 pesticides on dairy products to evaluate the method performance and verify its applicability for routine analysis. Five dairy products with different fat content were selected as representative of cheese category: yoghurt (fat content 10%); liquid cream (15%), goat cheese (20%), soft cheese (25%) and fat cheese (36%). The samples were extracted by acetonitrile (ACN), cleaned by C18 SPE eluted with ACN and analysed by triple Quadrupole GC-MS/MS technique. After extraction and clean up the residue in the final extract was in the range 19–37 mg (initial fat content 10–40%; residue in final extract <1%). In order to reduce the introduction of matrix constituent into the chromatography column, the final extract was diluted up to 2 mL with ACN, before injection in GC-MS/MS. Blank samples were analysed and in general no interferences with the selected compounds were observed. Recoveries at two levels (LOQ ≤ 0.005 mg/kg and 10xLOQ) were performed. The results obtained are within the criteria of acceptability of 70–120 % with RSD ≤20% for all pesticides on all matrices tested, with exception of buprofezin, deltametthrin, fenvalerate and propargite, that showed good recoveries only for matrices with fat content up to 20%. All validation parameters are evaluated according to the Document SANCO/12571/2013.

[1]: G. Amendola, P. Pelosi, D. Attard Barbini, J. Environ. Sci.Health., Part B (2015) 50, 109–120
[2]: P. Pelosi, G. Amendola, T. Generali and R. Dommarco. Poster at the LAPRW 8-11 May 2011, Montevideo, Uruguay

Keywords: *dairy products, pesticide residues, chromatography-mass spectrometry triple quadrupole detector (GC–MS/MS–QQQ).*

P39
LC–MS/MS METHOD FOR EU PESTICIDE MONITORING PROGRAMME

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In April 2015 the European Commission decided the realization of a multi-annual control programme for 2016, 2017 and 2018 (REGULATION (EU) 2015/595). The aim of this directive is to monitor pesticides in different commodities of plant and animal origin. We developed a method for the determination of the pesticides mentioned in this regulation that are amenable to Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS) and focused on products of plant origin. The QuEChERS-methodology (Quick, Easy, Cheap, Rugged and Safe) which is widely used for extraction and sample cleanup in pesticide residue analysis was used for the extraction of the samples. The need for high sensitivity in pesticide analysis requires the use of sophisticated, robust and sensitive MS systems that are able to detect the analytes to 10 pbb level or even lower The LC–MS/MS analyses were done with a highly sensitive Qtrap® 6500+ instrument using fast pos/neg switching. For further confirmation of the analytes some samples were run in MRM–IDA–EPI mode. The acquired spectra could easily be compared to library spectra by the use of the Peakview®/MasterView® software.

Keywords: *LC–MS/MS, pesticides, quantitation, qTRAP, pos/neg switching*

P40
ULTRA-SENSITIVE AND RAPID ASSAY OF NEONICOTINOIDS IN HONEY BY UHPLC–MS/MS

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Neonicotinoids are a class of insecticides widely used to protect fields (corn, canola, soybeans…) as well as fruits and vegetables. Their systemic distribution with high efficiency against sucking insects and long residual activity made them very popular within the global pesticide market. Recently the use of these compounds became very controversial as they were pointed as one cause of the honeybees colony collapse disorder. Since pollination is essential for agriculture, extensive studies have been conducted to evaluate the impact of neonicotinoids on bee health. Following this the European Food Safety Authority limited the use of thiamethoxam, clothianidin and imidaclorpid. Some European countries have banned or restricted the use of neonicotinoids. In order to better understand the effect of these compounds on bees and their contamination in pollen and honey, a highly sensitive assay method was necessary. A method was then set up to fulfill the following requirements: easy sample preparation, high recovery, high sensitivity and speed of analysis. Acetamiprid, chlothianidin, thiamethoxam, imidacloprid and thiacloprid were selected as target compounds. Thiamethoxam-d3, imidacloprid-d4 and chlothianidin-d3 were used as internal standards. The sample preparation method used was QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) with an additional dispersive solid phase extraction step. The mobile phase was optimized to offer the highest mass spectrometry ionization efficiency possible. With the selected mobile phases, a UHPLC method was set up to separate all compounds in less than 6 minutes. Since the final QuEChERS extract is organic, a POISE injection was used to obtain sharp peak shapes while preventing the need for an evaporation step. Finally a limit of quantification of 5 pg/g in honey was obtained for all compounds, which corresponds to a quantity of 5 fg on column. For some compounds, the signal to noise ratio obtained was large enough to foresee an even lower LOQ. Signal was linear up to 10 ng/g. Eight commercially available honeys bought at the local store were assayed using this method. They were chosen to be representative of the different qualities available for customers. The recovery (mixing extraction and matrix effect) was measured in all tested honeys and was ranging from 56 to 113%, if calculated on peak areas. Using the internal standard correction, recovery was ranging from 91 to 119%, in accordance with the guidelines. In all the tested honeys, 2 to 3 different neonicotinoids were found at concentrations ranging from 0.0012 to 0.394 ng/g. Only clothianidin was never found. The method demonstrated its fit to quickly assay neonicotinoids in honey at very low levels far under the regulated limits using novel ultra-high sensitivity LC–MS systems.

Keywords: *neonicotinoids, honey, high sensitivity, UHPLC–MS/MS*

P41
SCREENING AND CONFIRMATION OF MULTI PESTICIDE RESIDUE IN RED CHILI USING ALL IONS MS/MS FEATURE ON AN AGILENT 6530 LC/Q–TOF HIGH RESOLUTION MASS SPECTROMETER AND QUECHERS SAMPLE PREPARATION TECHNIQUE

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A fast and sensitive method has been developed for the analysis of ~140 pesticides in Dry (Red) Chili powder using the QuEChERS sample preparation technique and All Ions MS/MS feature using an Agilent Q-TOF LC/MS. All Ions MS/MS is a technique which is available for Agilent high resolution TOF and QTOF LC/MS Instruments. All Ions MS/MS uses the Agilent proprietary Personal Compound Database and Libraries (PCDLs) to identify compounds with information from their molecular ion and fragment ions. The PCDLs are content rich and include accurate mass information for thousands of compounds. Sample preparation was optimized in order to eliminate pigments and other co-extracts which can cause shift in the retention times, affect chromatographic peak shape and cause loss of sensitivity of the target analytes. The graphitized carbon black (GCB) which adsorbs colored pigments was used as a sorbent and primary secondary amine (PSA) for the effective cleanup of the acet onitrile extracts. The optimized sample preparation method involved extraction from 2 g of sample with 10 mL of water and 10 mL of acidified acetonitrile in the presence of sodium acetate buffer (1.5 g) and magnesium sulphate (6 g) (Agilent P/N5982-5755). Cleanup of the acetonitrile extract was achieved using 50 mg PSA and 50 mg C18, 7.5 mg GCB and 150 mg of magnesium sulphate (Agilent P/N 5982-0028). Screening and confirmation was done using Agilent's MassHunter Qualitative Software with All Ions MS/MS and accurate mass from QTOF. The spectra of each analyte were set up using the Agilent Personal Pesticide Database Library (G3878-6005). Target analyte MS/MS transitions and other parameters were selected by exporting the processed data in MassHunter Qual as Compound Exchange Format (CEF) file to MassHunter Quant. The quantification of the residues was performed using matrix matched standards prepared in residue free organic red chili powder. As the limit of quantification (LOQ) for most of the pesticides was ~10 ng/mL and was below their respective EU MRLs, the calibration was constructed between 5 ng/mL and 250 ng/mL. Chromatographic and mass spectrometric parameters were optimized to get the best sensitivity. The method was applied for the analysis of Red Chili powder samples and it was found that the method was suitable for the routine analysis of ~140 target analytes evaluated in this study.

Keywords: *pesticides screening, all ions MS/MS, Q–TOF, QuEChers, chili*

P42
AUTOMATED SCREENING FOR HUNDREDS OF PESTICIDE RESIDUES USING A GC–Q/TOF PESTICIDE ANALYSER

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For the analysis of volatile pesticide residues in foods, most laboratories have implemented GC/MS/MS methods because of the very high sensitivity and selectivity of these instruments. Some laboratories even monitor several hundred compounds using this technique. But GC/MS/MS is a targeted approach and only those compounds on the target list will ever be found. There is a need for a pesticide screening method that can look for a much larger number of analytes A GC combined with high resolution accurate mass (HRAM) quadrupole time-of-flight MS (GC–Q/TOF) detection, offers far more selectivity and sensitivity. When run in the TOF mode full HRAM spectra are acquired over the instrument's full mass range. Identification of analytes is possible when two or more characteristic accurate mass ions, together with their correct isotope ratios and spacing are found at the correct retention time. In theory, one could screen for an unlimited number of compounds so long as characteristic accurate (or exact) mass ions and retention times are known for each. This presentation will introduce GC–Q/TOF Pesticide Analyzer for the screening of pesticide residues in various foodstuffs using a GC–Q/TOF and electron impact ionization in combination with a retention time locked GC method, backflush for increased method robustness and a new accurate mass spectral database of pesticides. The complete method is directly installed, implemented and checked out on the system prior to the install of the system in the lab, dramatically reducing the time until the first real sample can be measured, without compromising the flexibility or sensitivity of the system.

Keywords: *GC–Q/TOF, high resolution, pesticide screening*

P43
SIMULTANEOUS FULL SCAN AND MS/MS ANALYSIS USING LC–TOF–MS FOR THE DETECTION AND IDENTIFICATION OF PESTICIDE RESIDUES IN FRUIT AND VEGETABLES

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Accurate mass spectrometers operated in full scan mode have some very important advantages over triple quadrupoles (e.g. optimisation of acquisition parameters for each compound in not required, in a single run unlimited number of compounds can be registered). However full scan mode not always is able to provide sufficient data for compound identification. It is common that only $[M+H]^+$ ion is detected and no other adducts nor in-source generated fragments are available. In these cases identification criteria cannot be fulfilled. That problem can be solved by using simultaneously full scan and MS/MS analysis. This combination can be realized with or without target list of analytes. All ions fragmentation (AIF) mode does not require predefined target list. In AIF full scan mode is interchanged with MS/MS. However in MS/MS quadrupole does not select any specific precursor, all ions undergo fragmentation with predefined collision energy (or energies). By that fr agments of all matrix compounds and alnalytes present in the sample are obtained. Thus obtained spectra are complicated. An alternative for AIF is Auto MS/MS. In this mode a list of targeted precursor ions is required. In Auto MS/MS spectrometer works in full scan mode, when an ion from the target list is detected then the instrument uses qudrupole to select this specific ion. Subsequently the ion is fragmented in the collision cell and obtained fragments are analysed. Because of precursor selection obtained spectra are easy to interpretation. This work describes the capability of LC-QTOF-MS for the simultaneous screening and identification of pesticides in fruit and vegetable matrices by use of: 1) All Ions MS/MS and 2) Auto MS/MS. In the All Ions MS/MS technique, data were acquired using low collision energy (0 V that provides MS information) and two high collision energies (10 and 20 V) to obtain MS/MS fragment information. Optimal collision energies for Auto MS/MS were selected from a commercial compounds library. The study was performed using QuEChERS extracts of tomato, zucchini and orange. The extracts were spiked with 125 pesticides and two concentration levels (0.010 mg/kg and 0.100 mg/kg) were evaluated. A dilution factor of five times was applied before injection. The results showed similar identification capabilities for two acquisition modes with more than 99% and 95% of the pesticides detected and identified at 0.100 mg/kg and 0.010 mg/kg respectively in tomato and zucchini. In orange matrix, identif ied pesticides were over 95% and 88% at 0.100 mg/kg and 0.010 mg/kg respectively. In "difficult" matrices such as orange, identification by Auto MS/MS is more confident. The observed false negative results were mainly as a consequence of a lack of compound sensitivity caused by ion suppression effects. Additionally a few cases of software failure were observed.

Keywords: *pesticide residues, accurate mass spectrometry, QTOF, MS/MS*

P44
ULTRASOUND-ASSISTED EXTRACTION OF PESTICIDE RESIDUES IN HONEYBEES AND DETERMINACION BY MICROFLOW–LC–MS/MS AND GC–MS/MS METHODS

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The value of the pollination in the world is incalculable. As an example, in some specific regions of North America, East Asia and Europe, it can be as high as $1,500 US dollars per hectare and in the EU its estimated value is around 22 euros billion annually, of which 3,292 euros million correspond to Spain. Different factors such as parasites, industrial agriculture, climate change and pesticides have been identified as responsible for bees and other pollinators decline. Among the pesticides, insecticides in particular present the most direct risk to pollinators. Although the relative role of insecticides in the global decline of pollinators remains poorly characterized, it is becoming increasingly evident that some insecticides, at concentrations applied routinely in the current chemical-intensive agriculture system, exert clear, negative effects on the health of pollinators – both individually and at the colony level. This work involves the ultrasound-assisted extraction (UAE) of pesticide residues (286 pesticides) in the whole honeybee (without previous crushing) using acetonitrile as solvent. In order to reduce matrix effect a dispersive- solid phase extraction (d-SPE) step using PSA and zirconium dioxide as sorbents was applied. The main parameters affecting the UAE method (amplitude of the ultrasonic probe and both number and time cycles) were optimized using an experimental design. Two multi-residue methods using microflow-liquid chromatography system coupled to a triple quadrupole mass spectrometer (microflow–LC–MS/MS) and gas chromatography coupled to a triple quadrupole mass spectrometer (GC–MS/MS) were the analytical tools for the quantitative analysis of pesticide residues. For the most pesticides, recoveries at concentration levels of 10 and 50 μg kg^{-1} were within the range of 70–130 % with a relative standard deviation (RSD) < 25%. The methods were applied to determine pesticide residue levels in twelve honeybee samples collected from Spanish apiaries, containing pesticide residues at levels higher than theirs quantitation limits (between two and four pesticides) in seven of the analyzed real samples. The highest pesticide residue levels were found for metalaxyl (146 μg kg^{-1}), acrinathrin (570 μg kg^{-1}) and pyridaben (3,900 μg kg^{-1}).

Keywords: *pesticide residues, honeybees, microflow-LC–MS/MS, GC–MS/MS, ultrasound-assisted extraction (UAE)*

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P45
QUANTIFICATION OF PESTICIDES IN FOOD BY POSTCOLUMN INFUSION OF MONITOR SUBSTANCES

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In the multi-residue analysis of pesticides in food, matrix effects (ME) are limiting factors when using liquid chromatography combined with electrospray ionization mass spectrometry. They are induced by co-eluting matrix compounds. To overcome these ME, often, the standard addition method is used. This method is currently the most effective way to compensate the influence of matrix compounds on the measurement. But it is also laborious and expensive. For this reason it is important to find an easier method for compensating the ME. Based on the finding that, at the same retention time, the MEs in one matrix are similar for different pesticides with diverse physicochemical properties, there is a new approach to overcome the ME [1]. The permanent postcolumn infusion of a monitor substance with the same behavior like the pesticides made it possible to correct the signal suppression or enhancement induced by the matrix effect. We analyzed pesticides in curry by using the two-dimensional liquid chromatography. Hereby, a HILIC was coupled with an RP-column. The sample preparation included only the QuEChERS-extraction [2]. A mix of the veterinary drugs dapson and ronidazol and of the isotopically marked standard diuron-d6 was used for postcolumn infusion. Signal intensities of all analytes were corrected for the ME recorded by the monitor substances. We were able to analyze the three neonicotinoides Acetamiprid, Imidacloprid and Thiamethoxame in spiked samples and in real samples with a variance under ±25% from the reference. This method is able to compensate the influence of matrix effects but not the losses of analytes by sample preparation. It might be possible to shorten the testing times and reduce lab costs by replacing the standard addition method by our approach for a reduced spectrum of analytes. This method presupposes that there are recovery rates that correspond to the SANCO criteria and a ME under 60 %.

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Keywords: *pesticides, matrix effects, post column infusion, two-dimensional LC-MS/MS*

P46
DETERMINATION OF POLAR PESTICIDES AT OFFICIAL CONTROL IN THE CZECH AGRICULTURE

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Polar pesticides represent a group of polar or highly polar compounds of which determination usually requires application of specific analytical procedures termed as single residue methods. The QuPPe method firstly introduced in 2009 by European Reference Laboratory is a complete analytical approach officially recommended for determination of polar pesticide residues and their metabolites such as glyphosate, perchlorate, fosetyl-Al and others. Polar pesticides as chlormequat and glyphosate belong to the most widely used plant protection products worldwide (they are at the first places in consumption in the Czech Republic). Plant growth regulators as chlormequat, mepiquat and ethephon are used in cultivation of cereals, oilseeds, pulses, fruits and vegetables or ornamentals. Glyphosate is the mostly commonly applied, broad-spectrum, systematic herbicide which is used in agriculture with genetically-modified glyphosate-tolerant crops, but it is also used in yards, gardens and other non-agricultural areas. Determination of these analytes is complicated due to their polar character and low molar mass. QuPPe-method based on extraction into acidified methanol and followed by a direct analysis of raw extracts by LC–MS/MS was established in our institute and validated for four named pesticides in feed and plant materials. Chlormequat and mepiquat are separated on a HILIC column and quantified by solvent-based calibration standards with the help of isotopically labelled analogues of the target analytes. Glyphosate and ethephon are separated on a Hypercarb column and quantification is performed by the standard addition approach. More details about the extraction method, the final analysis, the quantification approaches and the validation data will be discussed in the poster. Central Institute for Supervising and Testing in Agriculture is responsible for official controls of feedingstuff. Since 2012, the control programme has been extended by chlormequat and mepiquat determination in cereals, oil seeds and processed feed materials. This year, analysis of glyphosate and ethephon was introduced for the purpose of phytosanitary inspection conducted in order to identify the causes of plant damage. The results of feed official controls and phytosanitary inspection activities will be presented in the poster.

Keywords: *chlormequat, glyphosate, feed, QuPPe, LC–MS/MS*

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P47
OCCURRENCE OF PESTICIDE RESIDUES IN ITALIAN ORGANIC HONEY FROM DIFFERENT AREAS

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Agricultural contamination with pesticides is a challenging problem that needs to be fully addressed. Bee products, such as honey, are widely consumed as food and their contamination may carry health hazards. In this study 40 pesticides selected as representative of different contamination sources were measured in 40 honey samples using methods based on ASE extraction with clean up into the cell and GC–MS/MS detection (triple quadrupole). Particular emphasis was given to the pesticides utilised in intensive citrus orchards in order to elucidate and relate the honey contamination and its potential sources. Residues of many pesticides were found in most of the samples investigated. The majority of honey samples (94%) contained at least one of the pesticides even if their concentrations were found to be lower than its MRL. DDT, DDD and DDE were the compounds isolated with higher frequency in honey samples produced in the industrialised area. Chlorpyrifos and qu inoxyfen were the residues frequently detected in samples coming from the citrus orchard area. No residues were isolated in honey coming from the mountain area. The optimised extraction method showed good validation parameters in term of recovery, repeatability foe all compounds investigated. The results of this study show that pesticide contamination of honey is strictly related to the contamination source and could reflect the specific pollution of a given environment, confirming honey bee and beehive matrices as appropriate sentinels for monitoring contamination in the environment. This could represent an effective tool for beekeepers to select production areas especially for organic honey production.

Keywords: *pesticide residue, analysis, honey, acelerated solvent extraction (ASE), contamination sources, triple quadrupole (QqQ)*

P48
METHOD VALIDATION FOR SELECTED PESTICIDES IN POTATO BY GAS CHROMATOGRAPHY COUPLED TO SINGLE QUADRUPOLE AND TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Potatoes are an important staple food all over the world. To protect the crop from various diseases farmers apply a range of regulated pesticide formulations which can sometimes leave residues in the crop. To ensure that such residues do not constitute a health risk for consumers it is important to apply end control testing to agricultural products. As part of an initiative under the “Red Analitica de Latino America y el Caribe” (RALACA) network, the FAO/IAEA Food and Environmental Protection Laboratory contributed to the validation of a multi-residue method for 45 pesticides in potato using gas chromatography coupled to mass spectrometry (GC–MS). The method included the pesticides that are most frequently employed worldwide in agricultural production of potatoes. The aim of this study was to validate the method according to the Codex Alimentarius Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (CAC/GL 40-1993) and to compare the use of two different detection systems, namely a gas chromatograph coupled to a single quadrupole mass spectrometer (GC–MSD), and a gas chromatograph coupled to a triple quadrupole mass spectrometer (GC–MS/MS). ”In-house” method validation was conducted to provide evidence that the method is fit for purpose. The method performance was characterized in terms of its scope, specificity, accuracy, sensitivity, repeatability, intermediate reproducibility and robustness. The method was validated using two different sample preparation options. One was the original QuEChERS method using the Agilent QuEChERS kit combination salts (5982-5650 + 5982-5056), following the standard operating procedure outlined with these products. The second was an IAEA modified QuEChERS method using ethyl acetate as the extraction solvent and clean up using Agilent QuEChERS salts 5982-5056. The instrument acquisition method for the GC–MS/MS was taken from the Agilent’s Pesticide Analyser configuration based on a 7000C Triple Quadruple and a 20 minute constant flow set up with mid column backflush and a slight modification in the oven temperature programme, resulting in a 22.183 minute run. The instrument acquisition method and GC conditions were equivalent for the GC–MSD so that the performance of both technologies could be directly compared. The optimisation of the GC–MS/MS method included an assessment of the collision energies (CE) provided with the Agilent RTL MRM data base using potato matrix matched standards. Results from the method validation are presented for both detection instruments and extraction conditions. Both instruments had good sensitivity for a number of compounds; however the GC–MS/MS method is more robust due to the increased confirmatory power and lower variability in complex matrices.

Keywords: *pesticides, gas chromatography – tandem mass spectrometry, gas chromatography – mass spectrometry, potato*

P49
DEVELOPMENT AND VALIDATION OF AN EASY MULTIRESIDUE METHOD FOR THE DETERMINATION OF MULTICLASSES OF PESTICIDES IN POULTRY MUSCLE BY LC–MS/MS AND GC–MS/MS

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A multidisciplinary project entitled Safety of organic meat* in which we partner as the National Reference Laboratory for Pesticides in Food of Animal Origins and Commodities with High Fat Content, was implemented for addressing, among other actions, the chemical contaminant status of organic meat samples. In the course of this project, an analytical protocol for the analysis of 111 pesticides in poultry muscle by LC–MS/MS and GC–MS/MS was developed and validated according to the EU-SANCO guideline 12571/2013.The main objective of our work was to develop a sensitive and rapid method in order to analyze pesticides in almost 250 poultry muscle samples within in a limited time. QuEChERS approaches are known to be suitable for the multiresidues analysis of pesticides. To optimize a new QuEChERS protocol, 10 solvents and mixture of solvents, 3 types of salts and 5 different dSPE sorbents were tested. The choice of the most appropriate extraction solvent was critical due to the wide polarity range of targeted pesticides, Log(P) ranging from – 0.08 to 7.6 for fenthion oxon sulfone and permethrin respectively. Finally a mixture of acetonitrile/ethyl acetate (25/75) gave the best recovery rates. For the extraction and clean up steps, citrate and dSPE with Zsep+ (Supelco) were selected as the best compromise between recovery rate and matrix effects. Quantification by isotopic dilution was used. The limit of quantification ranged from 1 to 10 µg/kg in muscle with a median of 2 µg/kg. For pesticides analyzed by LC, the mean recovery rate was 74% with a RSDR at 16%. The developed method presents a good sensitivity and a wide polarity range of pesticides. This protocol was also applied to 39 pesticides analyzable by GC–MS/MS. The mean recovery rate for pesticides analyzed by GC was found at 87% with a RSDR at 11%.Work is in progress for the analysis of 250 poultry meat samples from conventional and organic farming. This new method could also be implemented in the framework of national and European official controls in order to analyze priority pesticides as boscalid, fluazifop-p-butyl, fluzilazole, haloxyfop and metaflumizone which are not in the scope of presently-available methods for food of animal origin.

Keywords: *organic food, QuEChERS method, multiclasses of pesticides, poultry muscle, LC–MS/MS and GC–MS/MS.*

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P50
SCREENING OF PESTICIDES IN LAKE VOMB USING LC–MS/MS, GC–MS/MS AND LC–QTOF AND CONSEQUENCES FOR DRINKING WATER MONITORING

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Introduction: Sweden has a decentralized drinking water supply system consisting of approximately 1750 drinking water treatment plants. The National Food Agency is regulatory authority and the inspections are carried out by the municipalities. Drinking water production in agricultural areas can be challenging from a quality perspective and the drinking water treatment plants have to rely on commercial laboratories for analysis of pesticides in raw water and drinking water. In Sweden, the sum concentration of pesticides in drinking water shall not exceed 0,5 µg/L and no single pesticide shall exceed 0,1 µg/L. However, the monitoring scope of the pesticides is not regulated.
Methods: In this study, pesticides in raw water, process water and drinking water were sampled using two types of passive samplers, Chemcatcher SDB-RPS Empore™ disc and Chemcatcher C18 EmporeTM disc. Samples were extracted with ethyl acetate and acetonitrile consecutively and extracts were thereafter combined and concentrated. The samples were thereafter analyzed using LC–MS/MS and GC–MS/MS according to the scope Sweden normally uses for control of pesticides in food, comprising approximately 400 compounds [1]. In addition, the samples were also screened using LC–qTOF [2].
Results: The extensive screening study identified 27 pesticides in raw water which all has been or is approved for use in Sweden. The most important finding was that only twelve of the pesticides identified are included in the scopes of the monitoring programs recommended for drinking water or raw water by some large commercial laboratories in Sweden. Of the pesticides identified in this study ten are still approved for use in Sweden and of those ten, only four are available in the monitoring programs recommended by the large commercial laboratories. Five pesticides were identified in drinking water at concentrations below the regulatory limits.
Conclusions: Broad screening of pesticides using passive samplers, LC–MS/MS, GC–MS/MS and LC–qTOF is an efficient tool to identify pesticides in order to improve and adjusted the monitoring programs. It is crucial to detect all pesticides present in the drinking water since the regulatory limit is based on a sum value for pesticides. Particularly for pesticides that are still approved, proper raw water monitoring is needed to detect trends at an early stage.

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Keywords: *drinking water, pesticides, passive samplers, LC-MS/MS, GC-MS/MS, LC-qTOF*

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P51
PESTICIDE RESIDUE AND FOOD COMMODITIES CLUSTERING BASED ON INTERNAL STANDARDS BEHAVIOR USING PRINCIPAL COMPONENT ANALYSIS

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The most common techniques for the analysis of pesticide residues in food samples are liquid chromatography (LC) and gas chromatography (GC), both coupled to mass spectrometry (MS). In the Laboratori de l'Agencia de Salut Pública de Barcelona (LASPB), more than 240 pesticides are daily analyzed in a wide range of food samples using these techniques. The quantification of pesticides is based on a calibration procedure with surrogate matrix matched standards (SMMS), which also includes the use of internal standards (IS). Some representative matrices are used to daily prepare the SMMS for the quantification of different sets of food commodities. Due to the huge number of combination of matrix-pesticides, it is extremely important to study the matrix effect. The aim of the work was to find correlation between food commodities / pesticides based on the response of the IS used, to establish a set of representative matrixes to ensure the correct calibration procedure. For that reason, eighteen compounds were tested as potential IS. The final aim was to select the best suited IS for each pesticide in each food commodity. First, the experimental conditions for LC–MS/MS and GC–MS/MS detection were established for the IS candidates and the compounds that did not show a proper behavior in both techniques were discarded. Then, the behavior of the IS candidates in 35 different sample matrices, including vegetables, fruits, nuts, honey, etc. was assessed. To handle and interpret all the results obtained, Principal Component Analysis (PCA) was applied to the complete set of data. The chemometric study allowed clustering sets of food commodities with similar behavior pattern and helped in the selection of the most suitable IS according to the pesticides to be determined and the sample matrices of interest. Finally, food commodities were grouped in 15 categories and 3 IS were selected.

Keywords: *pesticides, matrix effect, internal standards, surrogate matrix matched standards, principal component analysis*

P52
USING THE "NL"-EXTRACTION METHOD AND GC–MS/MS TQ WITH SHORT AND LONG RUN TIMES FOR LIMITED- OR EXTENDED-SCOPE ANALYSIS OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES

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Scopes of LC– and GC–MS/MS triple-quad (TQ) MS multiresidue methods can be adapted to the purpose of the analysis, in order to have short reporting deadlines with only a limited, priority scope for import control, or a more extended pesticides scope for monitoring purposes and complete consumer exposure calculations. Two GC–MS/MS TQ methods with different chromatographic run times (22 and 35 min) with a scope of 171 and 310 pesticides, respectively, were developed on a Bruker Scion GC–MS/MS TQ instrument, allowing measurement of 620 transitions during the 35-min long run time. Various GC– and MS-parameters were optimized in order to obtain the highest sensitivity. The two GC–MS/MS TQ methods were combined with the very fast and efficient Original and the New Dutch Mini-Luke extraction (NL-)method, without any cleanup required. A homogeneous 15 g sample is extracted with 20 mL acetone (30 sec via a Polytron homogenizer), followed by a partitioning step (again 30 sec via a Polytron homogenizer) with 20 mL petroleum ether, 10 mL dichloromethane and 15 g Na_2SO_4, using the salting-out effect in order to assure good recoveries for polar pesticides. The mixture is centrifuged (5 min at 3,500 rpm), an aliquot (3 mL) of the extract is then evaporated to dryness (batchwise, on a water bath) and the residue is redissolved in 0.9 mL isooctane/toluene (9:1) and 5 µL is directly injected (via LVI-PTV) into the GC–MS/MS TQ. Initial validation of the different methods was carried out for 171 and 310, for the original extraction method (short and long run, respectively), and for 131 representative pesticides, for the NL-method, (short run). The pesticides belonged to different pesticide groups and two representative matrices (lettuce and orange) were selected. Recovery studies were performed at spiking levels of 0.01 (or 0.005), 0.02 (or 0.01) and 0.05 mg/kg. Almost all analytes met the EU DG SANCO method validation criteria (i.e. average recoveries in the range 70–120%, with RSD <20%). Method LOQs were generally 0.005 mg/kg.

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Keywords: *GC-MS/MS triple-quad (TQ) MS methods, Pesticide residues analysis, "NL"-method (New Dutch Mini-Luke extraction method), Fruits and vegetables, Import control*

P53
EVALUATION OF MATRIX EFFECTS AND THE INFLUENCE OF SPECIFIC COEXTRACTIVES THROUGH LC(ESI) –HR–TOF OF ETHYL ACETATE EXTRACTS FOR PESTICIDE RESIDUES ANALYSIS IN DIFFERENT SPECIES AND VARIETIES OF CITRUS FRUITS

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Although pesticide residues analysis protocols within the citrus industry are well defined and applied all over the world, the determination is not an easy one. Citrus fruits and derivatives are complex matrices, and most analytes suffer an important Matrix Effect (ME). This phenomenon influence greatly the quantitation of the residues, and in extreme cases inhibit their determination. To avoid this problem, the SANCO guidelines [1] recommend the use of matrix matched curves for residues quantitation as well as it accepts that representative matrices of each group could be used for method validation. An investigation was conducted in order to evaluate the ME for lemon, mandarin and orange and different varieties including an ecological sample. The goal was to establish whether any matrix match calibration curve can be used to quantified pesticide residues in any other given citrus fruit and to characterize chemically the coextractives responsible for this ph enomenon. Eighty pesticides in the corresponding citrus blank extract obtained through the ethyl acetate (EtOAc) dispersive method [2] were evaluated using liquid chromatography coupled to high resolution time of flight mass spectrometry (LC–HR–ToF). The matrix matched and solvent curves were prepared in the range 10 to 150 µg/kg. The results showed that linear ranges are variable for each pesticide, where the main drawback of the method was the prompt saturation of the detector, due to some analytes and matrix components. Significant differences in the ME among lemon, orange and mandarin were observed, for example; acetamiprid showed 42% of ME in orange, 58% in lemon and only 5% in mandarin. Moreover, no significant differences in the residues quantitation were found when comparing varieties of the same species. The results show that the use of MM pesticide residues analysis calibration curves of the different citrus species has to be performed using the fruit species under study. The MM calibration curves for different varieties of either mandarins or oranges can be used for pesticide residue determination within the same species The chemical composition of the coextracted natural products was studied and 20 compounds were identified, either through comparing the HR-Mass spectra of standards or deduced form the fragmentation patterns. The pseudo-alkaloid synephrine, flavonoids such as naringin, neohesperidin, hesperidin, poncirin, sakuranin and fatty acids like oleic, linoleic and linolenic acid could be identified in the studied extracts. A principal components analysis including all the identified compounds, yielded clear distinction among the three species, but the varieties of mandarins as well as the oranges ones were grouped together.

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Keywords: *Citrus; pesticide; matrix effect*
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P54
PESTICIDE RESIDUE OCCURRENCE IN CZECH AGRICULTURAL SOILS

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Central Institute for Supervising and Testing in Agriculture ensures official controls and monitoring activities in various sectors of agricultural practise. Except for controls of feedingstuff and soil inputs the institute ensures the monitoring of physical, chemical and biological qualities of the agricultural soils in the network of permanent monitoring areas. The scope of monitored parameters in this programme was extended by currently used pesticides last year. These compounds are intensively used for plant protection at the conventional agricultural practise; however, there is little information available about their presence in the soil environment. An initial set of 60 compounds included the most frequently applied pesticides in the current agriculture as well as formerly used triazine herbicides. Samples were collected in two different sampling periods on 40 monitoring sites. Pesticide determination was carried out using the QuEChERS method based on acetonitrile extraction followed by phase partitioning step and final GC–MS/MS and LC–MS/MS analyses. Quantification was performed by matrix-matched calibrations. Applicability of the QuEChERS approach to soil samples, originally developed for pesticide analysis in plant matrices, will be discussed in the poster. Results showed that triazole fungicides and metabolites of triazine herbicides are the most frequently detected active substances in soil samples. Pesticide contents and profiles will be discussed with regard to aspects such as the stability of pesticides, the sampling period, the crop treatment methods and schedules. In addition, the pesticides profiles in agricultural soils will be compared to those found in crops and feed materials.

Keywords: *soil, pesticide, QuEChERS*

P55
OCCURRENCE OF MYCOTOXINS AND PESTICIDES IN MEDICAL PLANTS FROM RTANJ MOUNTAIN

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The mountain Rtanj is one of the most beautiful mountains in the southeastern Serbia. It belongs to the Carpathian mountains with the highest peak Šiljak at 1570 m above sea level. The northern part of the mountain is covered by woods and pastures of autochthonous species and abounds in fresh water springs. This mountain is known for its underground springs, caves, pits, medicinal plants, fire balls, which are a natural phenomenon, and other fascinating and unexplainable phenomena. Rtanj is known for its endemic plants that grow only on this mountain, and the most famous of them is certainly Rtanj tea (*Saturea montana*). Saturea montana is a perennial shrubbery plant 10–40 cm high with the lower trunks and branches lignified. The leaves are leathery and shiny covered in sparkling glands. The flowery tips are collected. The resources of this plant are significantly low in nature. That is why it is protected by law. Our study deals with mycotoxins and pesticide residues detection in Rtanj tea taken by random sampling at several market places from individual harvesters. The validated method based on the QuEChERS sample preparation for more than 70 target analytes (pesticides) with the determination by LC–MS/MS was used for the analyses of medical plant *Saturea montana*. The average recoveries for all analites were 67.9–117.4% (RSDs 4.27–18.19%). The detections of difenoconazole (0.46 mg/kg), cypermethrine (0.011 mg/kg) and propamocarb (0.013 mg/kg) exceeds the orientation value for pesticide residues in organic produced products of 0.01 mg/kg. The mycotoxin analyses involved the extraction of aflatoxin (AF) and ochratoxin-A (OTA) by methanol/water, immunoaffinity column (IAC) clean-up and HPLC quantification with fluorescence detection (FLD). In accordance with the obtained results it can be stated that the *Saturae montana* from the Rtanj mountain is safe concerning the mycotoxins.

Keywords: *pesticide residues, mycotoxins content, medical plant*

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P56
PESTICIDE RESIDUES IN FRUITS AND VEGETABLES FROM THE MACARONESIA (PERVEMAC): CANARY ISLANDS

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An intensive pesticide residue monitoring program is being carried out in the geographical area of Macaronesia, taking into account three different archipelagos: Canarias, Cape Verde and Madeira. This program is conducted in the framework of a Cooperative Research and Development Project, PERVEMAC, granted by the European Regional Development Fund (ERDF), under the European Territorial Cooperation programs. PERVEMAC was built on an unprecedentedly broad and comprehensive monitoring pesticide residues and mycotoxins in plant products, vegetal and cereals, consumed in the Macaronesia archipelagos. Sampling of vegetal products was made taking into account the pattern of annual vegetal consumption in each region, and the number of samples per year was decided on the basis of the number of inhabitants in each region: one vegetal sample per 2500 inhabitants and year: 800 samples/year for the Canary Islands. The present effort shows the results for the first six mo nths of the monitoring program developed in the Canary Islands, 387 samples of fruits and vegetables, 50% each, spanning 56 different commodities. The most sampled commodities were: apple (37), banana (68), orange (16), pear (14), lettuce (22), onion (10), pepper (20), potato (13), tomato (35), zucchini (12). The 387 samples collected are from local agriculture (239 samples, 62%) and importation (148 samples, 38%). Plant health legislation which regulates plant products may be imported into the Canary Islands and which are not, protect local production in the archipelago. In this regard, only a small number of commodities include a balanced number (minimum of 30% for lower) of samples between locally produced and imported samples: lettuce, orange and pepper were the most representative in this case scenario. The analysis of pesticide residues was carried out using two multirresidue methods, the first one using the ethyl acetate method followed by GCMSMS determination and the second one using Quechers followed by LCMSMS determination, addressing 85 and 45 different pesticides respectively. The dithiocarbamate residues were analyzed by the carbon disulfide method. The incidence of samples with pesticide residues was the 63% with a ratio of 1.68 residues/sample. The commodities with highest residue/sample ratio were pear (5.3 residues/sample) and table grapes (5.5 residues/sample). Looking for the comparison between the results for locally produced and imported samples, we find that for local samples the percent of samples with residues is 57% and the residues/sample ratio is 1.38, while for the imported samples these data are 71% and 2.16 respectively.

Keywords: *pesticide, residues, fruits, vegetables, Canary Islands*

Acknowledgement: *European Regional Development Fund (ERDF). European Territorial Cooperation programs: PCT-MAC*

P57
SHOOT-AND-DILUTE GC–MS/MS: USE OF SPLIT INJECTION FOR PESTICIDE RESIDUE SCREENING TO PROLONG GC INLET LINER AND COLUMN PERFORMANCE

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Shoot-and-Dilute GC–MS/MS uses split injection for GC paired with a very sensitive triple quadrupole mass spectrometer. Split injection can alleviate matrix related issues occurring at the GC inlet and column. There are well-known problems associated with splitless injection of dirty samples, most notably compound degradation and drastic response changes. This can occur very quickly with real samples, sometimes with a single injection of a particularly dirty sample. Inlet and column maintenance are needed to restore instrument performance resulting in instrument down time. An easy way to mitigate these problems is to use split injection when possible. That is, if limit of detection and limit of quantitation requirements are achievable using split injections at ratios of 10:1 or greater. Increased flow through the GC inlet with split injection minimizes residence time inside the inlet liner. This decreases compound degradation and maintains acceptable data quality longer, especially during dirty sample analyses. In addition to the benefits described above, the GC oven start temperature can be higher, thus reducing overall run time as well as the time needed to re-equilibrate the oven. Also, split injection of the common QuEChERS solvent, acetonitrile, is easily accommodated on a non-polar 5-type GC column allowing symmetrical peak shapes of early eluting compounds like methamidophos, dichlorvos, and acephate. This eliminates the need for extensive initial oven temperature optimization or time-consuming solvent exchange. Shoot-and-Dilute GC–MS/MS was tested for multi-class pesticides and compared with a splitless injection method. Split injection and initial GC oven temperature parameters were optimized. Viability of split injection based on detectability of over 200 analytes was determined in QuEChERS-prepared green bell pepper, celery, orange. Green bell pepper cleanup using dispersive SPE without graphitized carbon black sorbent was less than ideal with a relatively high level of chlorophyll remaining in the sample, which severely stressed GC inlet performance for splitless work while the split method was much more robust. Ruggedness of split and splitless methods was evaluated by comparing average response factors and corresponding % RSD, and by showing chromatographic performance differences, especially for compounds that show degradation products.

Keywords: *pesticides, GC–MS, GC–MS/MS, Shoot and Dilute GC, QuEChERS*

P58
SHOOT-AND-DILUTE GC–ECD FOR ANALYSIS OF PROBLEMATIC PESTICIDES

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In GC, most analysis problems occur at the GC inlet, where Captan and Folpet, e.g., can degrade during hot splitless injection, Omethoate can be adsorbed to liner surfaces, and dirty samples can compromise synthetic pyrethroids transfer from the inlet to the GC column. These issues are magnified due to the slow inlet flow during splitless injection, which is typically less than 2 mL/min. A way to mitigate these problems is to use split injection, what we term “shoot-and-dilute”, where the much higher inlet flow rate results in a substantially reduced residence time and a higher effective transfer for difficult compounds of interest. This technique is especially appropriate with ultra-sensitive detectors like the electron capture detector (ECD). This paper will demonstrate the use of split injection with an inlet liner specifically designed for accurate and repeatable transfer of pesticides with a wide volatility and chemical class range. “Shoot-and-dilute” fast GC–ECD for trace analysis of organochlorine pesticides will be demonstrated, while illustrating benefits such as increased system uptime, shorter analysis time, and higher sample throughput. Fortified EN Method QuEChERS strawberry extracts were used for both splitless and split injection. For the splitless work, by the end of 40 injections of strawberry extracts, Captan and Folpet had declined to less than 50% of their original responses, while pesticides like Bifenthrin had essentially the same responses. Deltamethrin substantially degraded to its earlier eluting isomer by the end of the splitless experiment. Quantitative accuracy suffers when this occurs. While split injection produces overall lower responses versus splitless injection, the ECD is sensitive enough to work below the typical MRLs for many pesticides, including Captan and Folpet. Captan especially is difficult to analyze using GC–MS anyway because it has very low-intensity higher *m/z* ions, which makes its quantification difficult to achieve without bias, even in samples that are not very complex. The same QuEChERS strawberry experiment for splitless was conducted for split (shoot-and-dilute) injection. At injection 40, Captan and Folpet responses were greater than 80% versus the original response. Over the course of 40 injections the average response factor % RSD values for Captan, Folpet, and Deltamethrin at 100 ppb in strawberry extracts were less than 6, while for splitless injection they were as high as 31 (Captan). Interestingly, the initial response factors (taking into account the amount on column), were higher in every case for the split injection, wh ich is a validation of the highly efficient transfer of pesticides when the flow rate is higher through the inlet.

Keywords: *captan, folpet, QuEChERS, GC–ECD, split injection*

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PROLONGING GC–MS/MS PERFORMANCE FOR PESTICIDE ANALYSIS: SHOOT-AND-DILUTE INJECTION AND ANALYTE PROTECTANTS

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In gas chromatography–mass spectrometry (GC–MS), most problems occur on the front end, at the GC inlet, where compounds can degrade during hot splitless injection, active compounds can be irreversibly adsorbed to inlet liner surfaces, and nonvolatile material from dirty samples can compromise the transfer of less volatile compounds of interest from the inlet to the GC column. These issues are magnified due to the very slow inlet flow during splitless injection, which is typically less than 2 mL/min. Two strategies to mitigate these issues will be demonstrated in this work. One approach is to use split injection, what we call, “Shoot-and-Dilute”. With newer, more sensitive GC–MS/MS systems, LOD and LOQ requirements are often achievable using split injections at ratios of 10:1 or even greater. Increased flow through the inlet during split injection minimizes residence time inside the inlet liner, which decreases compound degradation and adsorption, and maintains acceptable data quality longer. In addition, GC oven start temperature can be higher thus reducing overall run time as well as the time needed to re-equilibrate the GC oven prior to the next analysis. Another benefit of split injection is improved peak shape for early eluting pesticides when injecting acetonitrile-based QuEChERS extracts. A second strategy to overcome GC inlet problems is to use “analyte protectants,” which are essentially volatile and chromatograph-able masking agents such as sugars, diols, etc., that are co-injected with each sample and standard to temporarily occupy active sites in the GC inlet liner and column. These analyte protectants have low m/z ions and the mass spectrometer can essentially overlook them in favor of target compounds. Both strategies were tested with multi-class pesticides and compared against a typical splitless injection method without use of analyte protectants for QuEChERS samples. For Shoot-and-Dilute, viability of split injection based on detectability of a wide range of analytes was determined. Benefits of analyte protectants were evaluated by peak shapes and responses of both well-behaved and problem pesticides. The goal of both Shoot-and-Dilute and analyte protectants approaches is to improve initial and long-term chromatographic performance. There is even the possibility of using both together for an even greater benefit.

Keywords: *pesticides, split injection, analyte protectants, GC–MS/MS, QuEChERS*

P60
SIMPLE, RAPID EXTRACTION OF CHLORINATED PESTICIDES IN POULTRY TISSUE BY SOLID PHASE EXTRACTION AND GC/ECD

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Pesticide contamination is not limited to fresh produce; poultry tissue used for food consumption is also exposed to contaminants at levels that can pose harm to the human population. Pesticide extraction from poultry tissue using conventional liquid-liquid extraction (LLE) techniques can be time-consuming and create excess solvent waste. Additionally, the tissue matrix is complex due to the presence of proteins and lipids. Non-selective extraction methods such as LLE do not eliminate all interferences, and eventually result in decreased column lifetime and increased system maintenance. Presented is a simple, rapid solid phase extraction (SPE) method developed to selectively extract chlorinated pesticides from poultry tissue using minimal solvents, utilizing Strata® Alumina-N solid phase extraction cartridges. Following the extraction, GC analysis is performed using a Zebron™ ZB-MultiResidue™-1 column and electron capture detector (ECD). The column employs an extensively cross-linked stationary phase that offers selectivity necessary to separate structurally similar chlorinated pesticides. The optimized GC method results in a 19 min total run time for all the chlorinated pesticides, eluting all analytes within 15 min. Analytically, the method presents cleaner chromatograms that are free from matrix impurities and suitable for quantitative analysis. Comparatively, the SPE GC/ECD method for pesticide analysis from poultry tissue outperformed the traditional procedure with decreased laboratory space requirements, reduction of hazardous waste, and significant reduction of labor consumption, leading to greater overall laboratory productivity.

Keywords: *residues- pesticides, poultry, solid phase extraction, gas chromatography, food safety*

P61
A MULTI-RESIDUE ANALYTICAL METHOD FOR THE ASSESSMENT OF ORGANIC CONTAMINANTS IN HONEY

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Honey is produced by bees from flower nectar and honeydew and it has antiseptic properties, stimulates the immunological system and it is a source of many essential elements. Unfortunately, this food could be affected by several forms of contamination. The aim of this study was to set a new analytical method which allow to analyze simultaneously 67 contaminants belonging to different classes (PCBs, PAHs, organochloride, organophosphorus and other types of pesticides) by GC–MS/MS. The method was applied to analyze 47 samples of Italian honeys. The new extraction method consists in a liquid-liquid extraction with hexane and ethyl acetate and QuEChERS composed of $MgSO_4$ and NaCl. Analyses were carried out by a Thermo Scientific Trace GC Ultra coupled with a triple quadrupole mass spectrometer TSQ Quantum XLSwith SRM modality. Recovery values were between 80 and 137%, LODs ranged from 0.1 µg/L and 5 µg/L and LOQs between 0.3 and 15 µg/L. Intra-day instrumental repeatability was in the range 2.5–5.4%, whereas intermediate instrumental repeatability was between 3.7% and 9.0%. All the analyzed Italian honeys showed the presence of residues of organophosphorus pesticides. PCBs were below to the detection limits for all the analyzed samples; PAHs and organochloride pesticides were found in 46.8% and in 53.2% of the samples respectively. Moreover the samples were contaminated by piretroids and other types of pesticides. Finally, 46.8% of the samples exceeded the Maximum Residue Limits (MRLs) established for organic contaminant levels in honey by the European Community (Commission Regulation (EU) n°37/2010 and Regulation (EC) n°396/2005 and subsequent amendments). It is possible to hypotize that the extensive and undisciplined pesticide use during the last years caused the contamination of the wax and, consequently, the alteration of the honey, resulting in quality and healthiness concerns.

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Keywords: *honey, multiresidue analysis, QuEChERS, GC–MS/MS, organic contaminants*

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EFFECT OF KAOLIN CLAY TREATMENT ON PESTICIDE RESIDUE LEVELS IN TREE FRUITS

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Kaolin clay (kaolinite) is sprayed on apples and other tree fruits as a natural pesticide to prevent damage from Brown Marmorated Stink Bugs and a physical sun barrier to prevent sunburn and heat stress. Mineral clay has a long history of use for industrial and medicinal applications, of particular interest here as adsorbent for chemicals in water, and medical applications such as adsorbent for toxins produced by mold in corn. The hypothesis evaluated is whether the addition of clay to the pesticide treatment regimen of apples and peaches during production may have an influence on the levels of chemical pesticide residues in the fruit at the time of harvest. Apples and peaches from alternating rows having received the same pesticide treatments except for the use of kaoline clay were harvested at maturity. Sample preparation was performed using DisQue QuEChERS according to method AOAC 2007.01. Pesticide residues were measured on a Waters Acquity UPLC equipped with a Xevo-TQS triple quadrupole mass spectrometer. Results indicate that residues of cyprodinil, myclobutanil, thianethoxam, zinc dimethyldithiocarbamate, were reduced in fruits treated with kaolin clay, while acetamiprid, phosmet, pyraclostrobin abd trifloxystrobin were not reduced.

Keywords: *pesticides, clay, LCMSMS*

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P63
SIMULTANEOUS QUANTITATION AND CONFIRMATION OF ABOUT 500 PESTICIDE RESIDUES IN FOOD EXTRACTS USING LC–QTOF ACCURATE MASS SPECTROMETRY

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Fast and comprehensive full scan accurate mass screening and quantitation became an excellent tool in food control in particular if hundreds of pesticides have to be proved in a short time frame. Additionally to the high number of targets the technique takes advantage of unknown evaluation and retrospective analysis. However, practical studies of the performance of current high mass accuracy mass spectrometers reveal certain limitations. Due to matrix background and interferences from other pesticides, unambiguous identification and correct quantitation is a challenging task. This study evaluates the performance achieved with a high performance QTOF, new software developed for this purpose and a very high quality data base. For the study, a set of 500 pesticides was selected. The pesticides were mixed in a way to give similar response in the mixture. Matrix based dilution series (0.1–2,000 µg/kg, QuEChERS-extracts: tomato, summer squash, potato, orange) of the pesticide-mix were analyzed by an UHPLC-QTOF system using a 15 min gradient. Data acquisition is performed in alternating between full scan and bbCID all ion fragmentation mode. Automatic data evaluation is performed using TASQ, dedicated software for target analysis screening and quantitation. For automated confident identification we use RT, accurate mass, isotopic pattern and up to 3 qualifier ions in full scan and 7 qualifier ions in bbCID. As result of the study we determined statistics for the LODs and LLOQs of the 500 pesticides in the different matrices. For more than 95% of the pesticides we determined LODs between 0.5 and 5 µg/kg and LLODs between 1 and 20 µg/kg.

Keywords: *pesticide residues, high resolution QTOFMS, quantitation, software, multi-target-screening*

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P64
RECENT ADVANCES IN THE DEVELOPMENT OF CHOLINESTERASE BIOSENSORS AND THEIR APPLICATION FOR THE DETECTION OF PESTICIDE RESIDUES IN FOOD

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Pesticides (e.g. insecticides, herbicides, and fungicides) are widely used in agriculture to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity. Accidental release of pesticides due to spills, leaking pipes, underground storage tanks, waste dumps, and ground water may lead to their persistence in the environment for a long period of time. Due to the health hazards associated with pesticides, their residue limits in food, drinking water and environmental samples are subject of regulation and control and their rapid detection and reliable quantification has become increasingly necessary. Traditional analytical methods including gas chromatography, liquid chromatography, high-performance liquid chromatography, mass spectrometry, capillary electrophoresis have been developed for analysis of pesticides in contaminated samples. Although these methods have been used successfully for the detection of pesticides, they present certain limitations such as time-consuming sample preparation, requirement of expensive instrumentation, requirement of highly skilled personnel and complexity. For these reasons there is an expanding need for analytical methods able to provide simple, rapid, sensitive, selective, low cost and reliable detection of pesticides at low concentrations. Over the past decades, cholinesterase (ChE) biosensors have emerged as simple, rapid and ultra-sensitive tools for toxicity detection of pesticides in the environment, food and water. These biosensors have the potential to complement or replace the traditional analytical methods by simplifying or eliminating sample preparation and making field-testing easier and faster with significant decrease in cost per analysis. With the recent engineering of more sensitive ChE enzymes, the development of more reliable immobilization matrices and the progress in the area of microelectronics, ChE biosensors could become competitive for multi-analyte screening and could soon be used for the development of portable instrumentation for rapid toxicity testing of samples. In this paper, we will present a review of the recent advances in the fabrication of ChE biosensors and their possible applications for toxicity monitoring of organophosphate pesticide residues in food. The focus will be on the various immobilization methods used, the different strategies for the biosensor construction, the analytical performance of the biosensors and the advantages and disadvantages of these biosensor methods. The recent works done to improve the analytical performance, sensitivity and selectivity of these biosensors will also be discussed.

Keywords: *pesticides, Cholinesterase enzymes, biosensors, food, sensitivity*

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THE BENEFITS PROVIDED BY SELF-CLEANING MS ION SOURCE IN GC PESTICIDE RESIDUE ANALYSIS

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Analysis of pesticide residues represents one of the challenges in chemical food safety control. Since pesticides are a wide group of chemicals differing in physico-chemical properties, it is not possible to analyze them using one instrumental technique. In case of nonpolar and medium polar pesticides with a good thermostability, gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS) represents currently a ´gold standard´. In any case, the demand for low detection limits (LOD) is continuously growing, therefore, both sample preparation and instrumental parameters play an important role. That has to be considered within optimization process.
It should be noted that analysis of large series of food extracts may lead to deposition of sample dirt in GC–MS system including ion source. On this account, successive deterioration of system performance, including increased LODs, is typically observed. To avoid a time consuming system maintenance, self-cleaning ion source (SCIS) concept based on reduction of impurities by small amounts of hydrogen supply to the source has been introduced by Agilent.
In this study, a comprehensive investigation focused on the impact of SCIS on analytical parameters of multiresidue method has been performed. A wide range of complex food matrices spiked by 165 GC amenable pesticides was analyzed by GC–MS/MS employing SCIS operated under two tested modes: (i) on-line mode (activation of hydrogen supply during measurement) and (ii) off-line mode (activation of hydrogen supply after measurement).The results of 5 months study have shown that the application of the latter one has a potential to provide fairly better results in terms of pronounced improvement of analyte signals following the hydrogen cleanup. When using it after a set of ca 200 injections of food extracts, with analytes at lowest calibration level, the relative signal increase was observed in case of 75% of them. Regarding on-line cleaning, some interaction of hydrogen with detection process probably occurred, no unambiguous improvement of GC–MS/MS system performance was observed.

Keywords: *self-cleaning ion source, GC–MS/MS, pesticide residue*

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CHALLENGES IN ANALYSIS OF PESTICIDE RESIDUES IN DIFFICULT MATRICES

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Nowadays, “green” food supplements such as hops, green barley and chlorella are very popular due to a high content of protein, fats (mainly polyunsaturated fatty acids), carbohydrates, fiber, chlorophyll, vitamins, and minerals. Moreover, hops represent the main ingredient of beer which is the most favorite drink not only in the Czech Republic. The content of pesticide residues in these products is regulated by the directive EC 398/2005.
In this study optimization and validation of a method for determination of more than 250 pesticide residues in hops, green barley and chlorella was performed. Analysis of these “difficult” commodities is quite challenging because of a high content of co-extracts (chlorophyll, flavonoids, volatile oils, acids etc.) in final extracts. Commonly used procedures do not provide enough purified extracts required for the final instrumental analysis. For the isolation of target analytes, we decided apply a QuEChERS-based extraction method, commonly used for analysis of tea samples. Ultra-high performance liquid chromatography coupled with tandem mass spectrometry (U-HPLC–MS/MS) was used for separation and detection of target pesticide residues.
Since pH is an important parameter affecting the extraction efficiency, in case of hops samples modification of pH value prior extraction was tested: i) addition of 1% formic acid (pH 2), ii) addition of deionized water (pH 6), iii) addition of 1 ml of 1 mol·l^{-1} Na_2CO_3 (pH 10), iv) addition of 2 ml of 1 mol·l^{-1} Na_2CO_3 (pH 12). Neutral pH was chosen as optimal for most of the tested pesticides.
In the next step, a clean-up procedure based on freeze drying followed by dSPE using PSA as a sorbent was applied. Whereas in case of chlorella and green barley, purification was shown as a beneficial practice, for hops the final validation was performed without the clean-up step.
The final validated method was subsequently applied for analysis of a set containing 8 hops samples that were intentionally contaminated during a growing and for analysis of a set containing 5 chlorella and green barley samples from the Czech market.

Keywords: *pesticide residues, hops, chlorella, green barley, U-HPLC–MS/MS*

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LC–MS/MS ANALYSIS OF RESIDUES OF QUATERNARY AMMONIUM COMPOUNDS IN FOOD MATRICES: TROUBLESHOOTING ISSUES

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Pesticides based on quaternary ammonium salts are often used as plant growth regulators. They are registered for use on cereals, cotton and ornamental plants. As plant growth regulators, they inhibit cell elongation. By this, Theky effect shortening and strengthening of stems and producition of sturdier plants. Plant growth regulators also influence the development cycle, which may lead to increased flowering. They may also support chlorophyll formation and root development. Thanks to these effects, quaternary ammonium salts based pesticides are used to increase the yield, improve quality and alleviate stress-induced adverse effects on crops production such as wheat, rye, oats or cotton. Sometimes mepiquat and chlormequat are used to increae yield of fruit and vegetables. Difenzoquat is a selective, post-emergent herbicide used for control of grassy weed in cereals. On the other side, residues of quaternary ammonium salts in these commodities may pose a health risk to humans, especially to small children. For this very reason it is necessary to monitor the occurence of their residues in selected commodities. Maximum residue level (MRL) for mepiquat and chlormequat in fruits, vegetables, honey, eggs and poultry is 0.05 $mg.kg^{-1}$. For cereals it is 2 mg kg^{-1}. Quaternary ammonium salts are commonly determined by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Targeted analytes are extracted with 1% solution of formic acid in methanol. Analytes can be determined without problem in a fruits or vegetables by this procedure. For other matrices, it is necessary to modify the extraction process. The presented study is focused on the determination of chosen analytes mepiquat, chlormequat and difenzoquat as representative pesticides based on quaternary ammonium salts. Problematic matrices chosen for this study were wheat, honey, milk and beef meat. In wheat and honey the results accuracy is influenced by high saccharides content interfering with analytes determination. In a case of milk and beef meat the fats are the main interfering agent responsible for interfering with analytes determination.

Keywords: *chlormequat, mepiquat, difenzoquat, LC–MS/MS*

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P68
CURRENT CHALLENGES IN LC-MS MULTI-RESIDUE METHOD FOR DETERMINATION OF PESTICIDES IN TEA

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Processed tea leaves (*Camellia sinensis*) are used for preparation of one of the most popular beverages worldwide. To achieve higher quality and yield of tea, pesticides (mainly insecticides) are frequently applied during plant cultivation and storage.

The analysis of pesticide residues in tea is in a great extent hampered by major co-extracted matrix components, such as caffeine, polyphenolic compounds (e.g. catechins), pigments, *etc*. When using LC–MS with electrospray for analysis of pesticide residues in tea extract, an intensive suppression of analytes ionization is often encountered.

In this study, a method for the determination of pesticide residues in green tea was developed and validated. The samples were extracted by QuEChERS-based method. Various clean-up procedures, such as applications of different sorbents (PSA, GCB, C_{18}) and addition of drying salts ($MgSO_4$, $CaCl_2$) within the d-SPE or freezing out, were tested to reduce the amount of co-extracted matrix components. Identification and quantification of target analytes was performed by liquid chromatography coupled to tandem mass spectrometry performed on a triple quadrupole. With regard to a complexity of tea extracts (even when the clean-up step was applied), LC-gradient was optimized to achieve better separation of analytes from matrix co-extracts.

The final method employing QuEChERS-based extraction, clean-up by freezing out (2 hours) followed by d-SPE (with PSA and $CaCl_2$) and an optimized gradient program of LC measurement was validated. Green tea samples spiked at two concentration levels (20 and 200 µg kg^{-1}) were used for these purposes.

For 313 analytes, the recoveries in the range 70–120% were achieved and repeatabilities (expressed as a relative standard deviation) were typically <5%. The final method was successfully applied to determination of pesticide residues in real green tea samples.

Keywords: *pesticide residues, tea, matrix effects, clean-up procedures, LC-MS/MS*

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P69
LC–MS/MS DETERMINATION OF HIGHLY POLAR TOTAL HERBICIDE GLYPHOSATE IN POPPY SEEDS

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Pesticides are an integral part of today's agriculture practice. A wide range of substances with different physico-chemical properties and biological effects have been registered. In general terms, pesticides enable improving the effectiveness of agricultural production, some of them facilitate mechanized harvesting crops. In the latter case, total herbicide glyphosate (N-(phosphonomethyl)glycine) is often used for this purpose, legumes, poppy seeds and many other crops are treated with preparations containing glyphosate as an active ingredient. With regards to recent toxicological report by the International Agency for Research on Cancer (IARC) which concludes that the herbicide glyphosate is probably carcinogenic to humans, the health risk associated with glyphosate residues in human food chain has been widely discussed by both scientific community and regulators.
To control the content of glyphosate residue and residues of other highly polar pesticides, the aqueous methanol based extraction procedure called QuPPe (Quick Polar Pesticides) is often used. In this study concerned with analyzing oily matrix such as poppy seeds, the extraction procedure was modified by addition of hexane into extraction mixture thus enabling removing of interfering lipids by partition. Ion exchange chromatography (IEC) coupled with tandem mass spectrometry (MS/MS), triple quadrupole (QqQ), was employed for glyphosate quantification. Under these conditions the limit of quantification was 0.04 mg/kg, the average repeatability of measurements (expressed as relative standard deviation (RSD) in percent) was 10% and the recovery was 109% for 0.120 mg/kg concentration level and 112% for 0.4 mg/kg concentration level.
The set of examined poppy seeds consisted from 41 commercial samples, 12 of them contained glyphosate residues, all above a stringent EU maximum limits (MRL) 0.1 mg/kg, the maximum contamination level was 1.035±0.414 mg/kg.

Keywords: *glyphosate, poppy seeds, IEC, mass spectrometry, QuPPe*

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P70
PESTICIDE RESIDUES IN FRUITS AND VEGETABLES FROM THE MACARONESIA (PERVEMAC): CAPE VERDE

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PERVEMAC is a Cooperative Research and Development Project granted by the European Union, which was built on an unprecedentedly broad and comprehensive program of monitoring pesticide residues and mycotoxins in plant products, vegetal and cereals, consumed in the Macaronesian archipelagos. Sampling of vegetal products was made taking into account the pattern of annual vegetal consumption in each region, and the number of samples per year was decided on the basis of the number of inhabitants in each region: one vegetal sample per 2500 habitants and year. In the present effort we show the results for the first part, six months, of the monitoring program developed in Cape Verde, reaching a total of 92 samples for the first semester of sampling, corresponding to 18 different commodities: tomato (27 samples), banana (11), carrots (11), cabbages (12), pepper (6) were the most sampled ones, with 18 samples more of different fruits and 7 of different vegetables. The origin of the 92 samples was mostly from local agriculture, 80 samples, with 12 samples for imported items: apple (5), papaya (5), citrus fruits (4), watermelon (2), kiwi and carrots (2). The analysis of pesticides was carried out with two multirresidue methods, the first one using the ethyl acetate method followed by GCMSMS determination and the second one using Quechers followed by LCMSMS determination, addressing 85 and 45 different pesticides respectively. The dithiocarbamate residues were analyzed by the carbon disulfide method. The incidence of samples with pesticide residues was the 30% observing a 23% of the 80 samples from local agriculture with residues and the 83% of the imported ones with residues, pointing that the two carrot samples imported were clear of pesticide residues and the 100% of the imported fruits with residues. The presence of residues expressed as the averaged ratio of residues by sample reached the value of 0.80 residues/sample, showing dramatic differences between local and imported items: 0,45 and 3.17 residues/sample respectively for local and imported items. Taking into account the presence of residues for each different commodity, we have found two different case scenarios for local products: traditional (almost free of residues) and professional (greenhouses, highly productive) agriculture. Banana, papaya, cabbages, carrots, are crops traditionally open air grown in Cape Verde, while greenhouses of tomato, pepper and recently watermelon and melon are professional crops carried under greenhouses with harvest pointed mostly towards tourist facilities. Anyway, a low incidence of pesticide residues is observed for the local products in Cape Verde: 0.45 residues / sample. In the case of imported fruits, the incidence of pesticide residues reach values of 3.80 residues/sample, detailing 3.33 residues in the case of citrus fruits and 4.80 residues/sample for apples.

Keywords: *pesticide, residues, fruits, vegetables, Cape Verde*

Acknowledgement: *European Regional Development Fund (ERDF). European Territorial Cooperation programs: PCT-MAC*

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COST SAVINGS IN PESTICIDES RESIDUES ANALYSIS: MINI-QUECHERS AND GC/MS/MS WITH ULTRA-EFFICIENT IONIZATION

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Miniaturization of the QuEChERS extraction method along with the ability to inject lower sample amounts into the instrument results in substantial cost savings. A savings of 43-48% is realized due to using less solvent, sorbent and labeled ISTD. In addition, use of an ultra-efficient ionization source and only 0.5 µL injection are expected to reduce costs through decreased maintenance and also allowed for lower limits of quantitation (LOQ) determination. In this study, LOQs for 126 pesticide residues were established based on recovery from fortified carrot, tomato and celery matrices, all of which were purchased as organic. The AOAC QuEChERS method was proportionately scaled down such that 2g sample, 1 g salts and 2 mL acidified acetonitrile was used. The 2 mL dispersive tubes were Agilent Bond Elut dSPE and the resultant sample volume was 0.25 mL. Recovery was evaluated at 1, 5, 10 and 50 ng/g and assessed against post-spiked matrix matched calibration cu rves. A volume of 0.5 µL was injected and analyzed by EI-GC/MS/MS in selected reaction monitoring mode. Two 0.25 mm ID HP-5MSUI columns of lengths 5 and 15m, configured for backflush, were used. Recovery samples pre-spiked at 1, 5, 10 and 50 ng/g (n=6) were designated as quality controls and were quantitated against a post-spiked calibration curve. Calibration standards prepared at concentration levels of 0.5, 1, 5, 10 and 50 ng/g yielded correlation coefficient values (R2) that were ≥0.992 for 97% of the 126 pesticides used to fortify the carrot, tomato and celery matrices. Reported LOQs are according to SANCO/12571/2013 and based on six replicate recovery samples at a given concentration level for which average recovery falls in the range of 70 ≤≥ 120% and %RSD ≤ 20. LOQs of 5 ng/g or lower were reached for 95, 98 and 97% of the 126 pesticides analyzed in carrot, tomato and celery, respectively. An LOQ of 1 ng/g was reached for 86% of the pesticides in carrot, 89 % in tomato, and 90% in celery. Captan and folpet are base-sensitive compounds and often present issues in terms of recovery when using QuEChERS and precision during analysis. These challenging residues were quantified at 5 and 1 ng/g, respectively, by adding the commercially available ISTDs captan-d_6 and folpet-d_4 for only $0.04 per sample in comparison with $2.78 or $3.42 total cost per sample (depending on type of d-SPE). Significant cost savings were realized through both scaled down sample preparation and decreased instrument maintenance; this was made possible by using a miniaturized QuEChERS method and a high efficiency source for GC/MS/MS analysis.

Keywords: *Pesticides, Mini-QuEChERS, GC/MS/MS, savings, Agilent*

P72
DETECTION OF PESTICIDE BY ELECTROCHEMICAL TYROSINASE BIOSENSOR

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The use of pesticides, comes out some problems due to their toxicity, becomes inevitable to protect the agricultural product from pest and herb. Thus there is an increasing demand to develop fast methods for residue analysis. The enzymatic biosensors show remarkable advantage with respect to spectrophotometric and chromatographic techniques in order to determine the phenolic compound, genetically modified organisms (GDO) and especially pesticide residue, at low level. It is possible to detect the low level of analyte by enhancing the sensor signal. In this respect, the key factor is immobilization of enzyme to the appropriate support material. Recently magnetic nanomaterials are becoming the focus of researchers due to their special properties such as strong superparamagnetism, low toxicity and large surface area provides high enzyme loading. Some stabilizers (surfactants, metal nanoparticles and polymeric compounds) have been used to prevent aggregation of magnetic nanomaterials. Among them natural polymer “chitosan” possess several features including biodegradable, biocompatible, bioactive, nontoxic, film forming ability, physiological inertness and high mechanical strength. In this study, tyrosinase enzyme will be immobilized on magnetite (Fe_3O_4)-chitosan nanocomposite film. According to the preliminary data it is thought to be enhance the sensor signal, since Fe_3O_4 nanoparticles provide another pathway for electron transfer. The sensor components (chitosan film, Fe_3O_4 nanoparticles, Fe_3O_4 nanoparticle-chitosan support and tyrosinase-Fe_3O_4-chitosan nanobiocomposite film) will be characterized by AFM, FTIR and SEM to reliaze the surface morphology, binding mechanisms and surface image of each material respectively. The analytical performance of the developed sensor will be tested by using electrochemical measurement (cyclic voltometry and amperometry). Catechol will be used as substrate to monitor sensor signal (the electrochemical reduction of enzymatically produced product “o-quinones” to the catechol) as follows. catechol + tyrosinase (O_2) →o-quinone + H_2O (1) o-quinone + $2H^+$ +$2e^-$ → catechol (at electrode) (2). The detection of pesticide by developed enzyme sensor depends on decrease in sensor signal. This phenomena rely on the fact that the pesticide inhibit the activity of tyrosinase enzyme by preventing the formation of enzymatically produced o-quinones (cause decrease in number of electron) while catechol is consumed.

Keywords: *electrochemical enzyme sensor, magnetite nanoparticle, pesticide*

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P73
OCCURRENCE OF CLOMAZONE RESIDUES IN TOBACCO

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Clomazone [2-(2-chlorobenzyl) methyl-4, 4-dimethyl-3-isoxazolidinone], is a herbicide firstly produced in the early 1980s, which selectively blocks the synthesis of carotenoid and chlorophyll in weeds. Because of the broad-spectrum activity and low production costs, clomazone is frequently used against species of annual broadleaf weeds and grass in the different cultivations. Generally, the use of herbicides in growing tobacco is crucial to inhibit competitive weeds. Since herbicide residues in tobacco leaves cannot be totally removed during processing, the inhalation or exposure to tobacco smoke can result in them being drown in through skin or clothing. The prolonged and repeated exposure, endangers the health. The determination of its residues in tobacco is of great importance. In this study the method involves modified QuEChES method for the extraction of clomazone. The extracts were analysed by liquid chromatography tandem mass spectrometry (LC–MS/MS). LOD was calculated by MassHunter Qualitative Software and it was 0.005 mg/kg. The linearity was checked using matrix matched calibration from 0.005 to 0.100 µg/mL (corresponding to 0.025 to 0.500 mg/kg) with the R^2>0.99. The recovery data obtained by spiking blank tobacco samples at three concentration levels (0.025, 0.050 and 0.250 mg/kg) was in the range from 87.4–103.3% (% RSD 3.60–7.97%), except for the spiking level of 0.025 mg/kg the obtained recovery was 143.9%. The carbofuran-D3 was used as an internal standard. The analysis was done on 24 samples of dried tobacco leaves. The pesticide residues were not detected in 12 samples (50%). The remaining 50% of samples contained clomazone residues above LOQ (0.025 mg/kg), while in two samples the detections were above the guidance residue limit (GRL) and amounted to 0.24 and 0.30 mg/kg. The GRL suggested by CORESTA was 0.2 mg/kg. The maximum residue limits of pesticide residues in tobacco are not defined in the regulations e ither of our country or in the EU ant the USA.

Keywords: *clomazone, tobacco, QuEChERS, LC–MS/MS*

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DEVELOPMENT AND VALIDATION OF MULTIRESIDUE METHOD FOR THE DETERMINATION OF MULTICLASS PESTICIDE RESIDUES USING LC–MS/MS IN LEMONS

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The use of pesticides is widespread in citrus fruits production for pre- and post-harvest protection and many chemical substances may be applied in order to control undesirable moulds or insects. As a consequence, the monitoring of their residue levels in these products is a necessity. A validation study was conducted on a rapid multiresidue method for the determination of pesticide residues in lemons. In our study a liquid chromatography-tandem mass spectrometry multiresidue method, with a short sample preparation step, based on acetonitrile extraction (QuEChERS) is developed and validated according to the European Union guidelines such as SANCO/12571/2013 in lemons, with a large scope that includes pesticides of different chemical classes. The good sensitivity and selectivity of the method were obtained with the limits of quantification at 0.01 µg/kg. All pesticides had recoveries in the range of 70.4–118.9%, with relative standard deviation values less than 20%, at three investigated concentration levels (0.01 0.1 and 0.5 mg/kg). Some significant matrix effects observed for most of the tested pesticides were eliminated using matrix matched calibration. The excellent linearity of all studied pesticides was achieved with R2 ≥0.99 in lemon matrix. This validated multiresidue method was applied for the determination of pesticide residues in lemons (8 samples) and grapefruits (5 samples). The imazalil was detected in all analyzed samples, but with the detections below the maximum pesticide residues MRL (EU Regulation 395/2005).

Keywords: *pesticide residues, validation, LC–MS/MS, lemons*

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QUANTITATIVE AND QUALITATIVE CONFIRMATION OF PESTICIDES IN BEET EXTRACT USING HIGH RESOLUTION ACCURATE MASS (HRAM) MASS SPECTROMETRY

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As world agricultural trade has expanded and concerns over food safety have grown, the enforcement of stricter pesticide regulations has become of utmost importance. The European Union (EU) and the Japanese regulations are amongst the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficent, high-throughput screening and quantitation of multi-class pesticide residues. Here we will describe a methodology that will utilize high resolution accurate mass to quantify and confirm in a single experiment. 10 µL injections of extracted beet matrix containing many pesticides were injected onto C18 reverse phase column. Compounds of interest were eluted using a standardized gradient elution profile. Upon elution of the analytes, the MS valve was actuated to switch to waste therefore, keeping the MS source clean of matrix contamination issues during the wash step at the end of the run. A high resolution acc urate mass spectrometer with a heated electrospray source (HESI), was used to analyze the compounds of interest in positive ionization, and the data are collected, analyzed, and reported using a customized software. To test the assay, standard curves with seven points were prepared in beet matrix covering the range 0.01 µg/kg to 100 µg/kg. Quantitation was done on Full Scan parent ion while qualification was done by fragment ions (ms/ms) as well as spectra library matching. The calibration curves were linear over the ranges described above. The columns showed no deterioration in quality or performance when analyzing the beet matrix. Detection limits were well below the EU set MRL and the R^2 were better than 0.99.

Keywords: *quantitation, high resolution accurate mass, pesticide, pesticide explorer, spectra library*

RESIDUES

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VETERINARY DRUGS

(R1 – R41)

R1
ANABOLIC SUBSTANCES - DEVELOPMENT AND FULL VALIDATION OF TARGET MULTIRESIDUE ANALYSIS OF STEROID AND NON-STEROIDAL HORMONES IN ANIMAL FOOD ORIGIN BY UPLC/MS/MS

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Hormones are chemical messenger substances which convey specific signals for the regulation of endogenous processes (metabolism) within the body by specific receptors located in cells of target tissue and can be classified according to their chemical-physical structure (steroids, proteins etc.) and according to their function – metabolic effect (androgens, estrogens, gestagens etc.). The use of hormones in animal feed is not generally permitted within the European Union (EU). However, there is no ban in all countries. Currently, there are still insufficient data available to make a final assessment of health risks which could result from the consumption of the meat treated with such hormones. As a consequence, the analytical developments are driven towards ever lower detection limits, even in difficult matrices like meat, liver, fat and urine. Existing methods however, are unable to sufficiently achieve this. A new method testing different classes of hormones in meat, liver, fat and urine has been developed and validated. It is based on a simple and easy extraction/purification, followed by detection/quantification with UPLC/MS/MS triple quadrupole analyzer (MRM mode). Very high level of sensitivity were achieved (LOD=0,1–0,5 µg/kg) for all molecules concerned due to the resolving power of MS/MS working in MRM mode as well as in Q-Trap mode to obtain additional confirmatory information.

Keywords: *hormones, residues, animal tissues, urine, UPLC/MS/MS*

R2
EXPOSURE OF POLISH CONSUMERS ON VETERINARY DRUGS AND FEED ADDITIVES RESIDUES IN EGGS

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Eggs are used on a large scale in food industry, and it is important that they are free of undesired residues. The aim of this study was to evaluate the exposure of consumers on chemical residues in eggs. A number of 150 eggs, sampled by the Veterinary Inspection, collected in different part of Poland were screened for the presence of 120 analytes. Each sample was an average of 12 eggs. Samples were extracted using EDTA and 0.1% formic acid in acetonitrile:water mixture. After clean-up with zirconia coated silica cartridges, samples were analyzed using liquid chromatography-tandem mass spectrometry. The method performance characteristics fulfilled the criteria of Commission Decision 2002/657/EC. Out of 150 samples tested, 65 were contaminated mostly with traces residues of either veterinary drugs or feed additives. In 49 of samples coccidiostats such as salinomycin, dinitrocarbanilide, lasalocid, narasin and decoquinate were detected. However, only one sample with salinomycin at the level of 7.47 µg/kg was non-compliant (ML set as 3 µg/kg). The antibacterials: enrofloxacin, ciprofloxacin, doxycycline, trimetoprim and tylosin were detected in 23 samples. In one case it was antibacterial compound below the MRL value, and the others are forbidden for use in laying hens. Anthelmintics: albendazole (forbidden for use) and fenbendazole (below MRL) were detected in two samples. In total, 12 out of 150 samples were found non-compliant with the European and national criteria. The exposure was assessed for adults (70 kg b.w.) and children 1–3 years of age (12 kg b.w.) with the intake assumed as 100 g and 43 g of eggs, respectively. The worst case scenario evaluation (the highest concentrations detected in eggs taken for calculation) revealed that the exposure of Polish consumers exceeded ADI for none of the analyzed compound. The highest concentrations in eggs were detected for enrofloxacin (94.4 µg/kg) and doxycycline (118 µg/kg) and resulted in the adults' intake of 7% ADI and 5.6% ADI, respectively. Children would be more exposed; the intake would exceed 23% ADI for enrofloxacin and 19% for doxycycline. Polish consumers are sufficiently protected from contaminations like veterinary drugs and feed additives from source which is the eggs. However, ADI limits are usually calculated for individual compounds. Possible interactions between substances are not taken into consideration. The mechanisms that may occur in the human body after ingestion of eggs containing residue of several chemicals are not known.

Keywords: *residues, eggs, veterinary drugs, coccidiostats, illegal dyes*

Acknowledgement: *Part of the study was financed by: National Science Centre "Sonata" 2012*

R3
DETECTING B-AGONISTS TREATMENTS IN FOOD PRODUCING ANIMALS: AN OVERVIEW OF ANALYTICAL POSSIBILITIES

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B-agonist drugs are synthetic molecules used for therapeutic purposes in human as well as in veterinary medicine for their anti-asthmatic, bronchodilatator, tocolytic and cardiotonic properties. However, and despite their ban for growth promoting purposes [Dir 1996/22/EC], their illegal use in food producing animals is regularly suspected in Europe and in edible tissues imported from third countries. The presence of residues and their associated harmful effects on humans makes the control of such veterinary drug residues a key component in ensuring consumer protection [Dir 1996/23/EC]. The control of βb-agonists misuse received extra attention after outbreaks of food poisoning in 1990 in Spain caused by consumption of bovine liver. This was the first time that pharmacological residues found in slaughtered cattle were found to have caused acute intoxication in consumers. Although clenbuterol is still probably the most popular known βb-agonist illegally u sed in farm, a wide range of other β-agonists exists which constitutes a challenge in detecting their use. Besides classical protocols of administration, some practices consisting in the use of "cocktails" composed of mixtures of low amounts of several substances that exert a synergistic effect and exhibiting similar growth promotion properties have also been reported as additional challenge to be overcome by control laboratories [Courtheyn et al. 2002]. This talk will overview the various possibilities to detect the administration of βb-agonists in meat producing animals. The choice of biological matrices of interest (meat, liver, lung, retina, urine...) together with the selection of the most appropriate analytical strategies (targeted, effect based approaches...) will be discussed, considering screening and confirmatory requirements.

Keywords: *b-agonists, chemical food safety, screening / confirmation, targeted anaytical strategies, untargeted analytical strategies*

R4
DEVELOPMENT OF A NEW MULTIRESIDUE TARGET APPROACH: ANALYSIS OF BETA AGONISTS IN LIVER USING TURBOFLOW TECHNOLOGY COUPLED WITH UHPLC/MS/MS

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Beta agonists (Beta androgenic receptor agonists) are bronchodilators that open air passages by relaxing the tightened muscles surrounding the bronchial tubes. These drugs are commonly prescribed to treat pulmonary diseases such as asthma, emphysema, and bronchitis or as a therapeutic treatment of tocolysis for female cattle during calving and for horses not raised for human consumption. European legislation forbids the use of beta-agonists as growth-promoting substances in cattle raised for human consumption. However, the beta-agonists are being used worldwide as illegal growth promoters in meat production, since they lead to higher total red meat yield. Use of a method multiresidue target analysis for detection and quantification of beta agonists is driven by market expectations. However, this is difficult to achieve for complex matrices such as liver. A new method to test beta agonists in liver has been developed. It is based on simplified extraction procedure, followed by on-line purification/detection/quantification using TurboFlow Technology – UHPLC/MS/MS triple quadrupole analyzer (MRM mode). Very high level of sensitivity were achieved (LOD = 0,1–0,5 µg/kg for all the molecules concerned) due to the power of MS/MS approach in MRM mode and due to purification capabilities of the TurboFlow technology, which lead to a reduction of matrix effects as well as to reduction of sample preparation time.

Keywords: *beta agonists, liver, residues, TurboFlow, UHPLC/MS/MS*

R5
EMR-LIPID: HIGHLY SELECTIVE MATRIX REMOVAL FOR MULTI-RESIDUE ANALYSIS IN COMPLEX SAMPLES

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Current methodologies for multi-residue analysis often implement a general extraction followed by analysis with a selective instrument such as LC/MS/MS, GC/MS/MS, GC/MS, etc. Although these techniques are simple and ideal for the extraction of diverse analyte groups, they also extract a large amount of matrix and traditional cleanup sorbents struggle to effectively and selectively remove these unwanted interferences. Complex samples high in lipids are particularly problematic as co-extracted matrix can cause poor reproducibility, ion suppression/enhancement, changes in analyte response over time, and more instrument maintenance. Agilent Bond Elut Enhanced Matrix Removal – Lipid (EMR-Lipid) represents the next generation of sample preparation technology; providing selective lipid removal for complex samples without analyte retention. EMR-Lipid is available in a convenient dispersive solid phase extraction (dSPE) format and is amenable to widely accepted workflows such as QuEChERS and protein precipitation. Data will demonstrate the performance benefits achieved by cleaner sample extracts using this new material in applications involving multi-class, multi-residue analysis for pesticides, veterinary drugs, and mycotoxins in complex, high fat samples. Dramatic improvements will be shown for matrix removal, analyte recovery, and reproducibility compared to currently available cleanup sorbents. The high performance and selectivity of EMR-Lipid make it an attractive option for laboratories seeking to simplify sample preparation for fatty samples, while enhancing analytical and instrumental integrity.

Keywords: *lipid removal, mycotoxin, veterinary drugs, pesticides, residue analysis*

R6
DISPERSIVE SOLID PHASE EXTRACTION FOR QUANTIFICATION OF ANTIBIOTIC RESIDUES IN DAIRY PRODUCTS USING UHPLC–MS/MS

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Public food safety scandals are increasing the uncertainty among the customers concerning the food safety and the resulting healthy risks. Such scandals often lead to significant adverse economic effects on food production industry, whereas it is quite understandable that manufacturers want to be unique with their products on the market and offer the best and safest products to the customers. Nevertheless, the high consumption of dairy products requires an intensive raw milk production. To avoid any economical drawbacks, antibiotics are administered to dairy cattles for the treatment and prevention of diseases (e.g. mastitis, diarrhea, pulmonary diseases) as well as for increasing the milk production. This prevalent use of antibiotics is a potential source of residues which can be taken up by human organisms through dairy products. Additionally to undesirable side effects like allergic reactions, the highest concern for consumers as well as for animal livestock is the development of a worldwide antimicrobial resistance, (e.g. MRSA - methicillin-resistant Staphyloccocus aureus) due to misuse and overuse of antibiotics. To protect the customers the European Union set maximum residue levels (MRLs) in the commission regulation (EU) No. 37/2010 for pharmacologically active substances in animal derived food stuff. In accordance to these MRLs, this work aimed to develop a confirmatory UHPLC–MS/MS multi-class method for quantitative determination of antibiotic residues in milk and dairy products (e.g. whipped cream, butter, cheese etc.) involving over 20 substances from five different substance classes of antibiotics, i.e. quinolones, macrolides, lincosamides, sulfonamides and tetracyclines. Additionally, the sample extraction and clean-up step should be easy to handle and possible for routine use to ensure a high sample throughput. QuEChERS based approaches [1,2,3] were conducted in previous experiments, resulting in non satisfactory recovery rates for the compounds of interest in milk. Therefore, sample preparation with C18 bulk sorbents as a combination of the easy and fast sample preparation provided by QuEChERS and the clean up principle of reversed phase SPE was investigated. In expectation to reduce matrix interferences more effectively these experiments were additionally carried out with C18/ZSep bulk sorbents to eliminate sugar, lipids and proteins from the samples. Results showed mean recoveries of the investigated substances in nearly all matrices ranging from 60–130%. Only tetracyclines indicated mean recoveries up to 180% maybe explained by their typical chelate formation. Problematic matrices seem to be fatty cheese as well as dairy products with higher protein and carbohydrate content, which has to be eliminated by further clean-up steps.

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[2] Stubbings G et al. (2009) Analytica Chimica Acta 637: 68-78
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Keywords: *antibiotics, dispersive solid phase extraction, HPLC–MS/MS, bulk sorbents*

R7
NON-ALLOWED PHARMACOLOGICALLY ACTIVE SUBSTANCES: EFSA'S WORK ON RPA, CHLORAMPHENICOL AND NITROFURANS

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Veterinary medicinal products (VMPs) may only be placed on the market if the residues in animal products do not pose any harm to the consumer. Pharmacologically active substances fulfilling this condition are classified as 'Allowed substances'. Regulation (EC) No 470/20094 stipulates that for non-allowed pharmacologically active substances a reference point for action (RPA) may be established. When residues of such non-allowed substances are detected at or above the RPA, the food is considered not to comply with Community legislation, and should be removed from the market. The European Food Safety Authority (EFSA) was asked by the European Commission (EC) to develop a guidance document on methodological principles and scientific methods to be taken into account when establishing RPAs for non-allowed pharmacologically active substances in food of animal origin. The Panel on Contaminants in the Food Chain (CONTAM Panel) developed a simple and pragmatic approach which takes into account both analytical and toxicological considerations. In addition, circumstances were identified under which the EC might consider it appropriate to consult EFSA for a substance-specific risk assessment; including substances causing blood dyscrasias (such as chloramphenicol) or that are high potency carcinogens (such as nitrofurans). As chloramphenicol and nitrofurans are excluded from the RPA guidance document and taking into account the presence from sources other than use of VMPs, the EC asked EFSA for scientific opinions on the risks related to the presence of chloramphenicol and nitrofurans. Chloramphenicol is implicated in the generation of aplastic anaemia in humans and causes reproductive/hepatotoxic effects in animals. The CONTAM Panel concluded that it is unlikely that exposure to food contaminated with chloramphenicol at or below the RPA of 0.3 µg/kg is a health concern for aplastic anaemia or reproductive/hepatotoxic effects. Chloramphenicol exhibits genotoxicity but, owing to the lack of data, the risk of carcinogenicity cannot be assessed. The CONTAM Panel concluded that, when applied to feed, the current RPA is also sufficiently protective for animal health and for public health, arising from residues in animal derived products. Nitrofurans are rapidly metabolised, occurring in animal tissues as protein-bound metabolites. Nitrofurans and their marker metabolites, generally, are genotoxic and carcinogenic and, also, have non-neoplastic effects in animals. Based on the calculated MOEs, the CONTAM Panel concluded that it is unlikely that exposure to food contaminated with nitrofuran marker metabolites at or below 1.0 µg/kg is a health concern.

Keywords: *non-allowed pharmacologically active substances, reference point for action, nitrofurans, chloramphenicol, MOE*

Acknowledgement: *EFSA wishes to thank the members of the EFSA SWG on non-allowed pharmacologically active substances in food and feed and their reference points for action and the members of the former Panel on Contaminants in the Food Chain (CONTAM Panel 2012-2015 mandate, http://www.efsa.europa.eu/en/contammembers/contampreviousmembers.htm). EFSA and the CONTAM Panel acknowledge all European competent institutions that provided occurrence data on chloramphenicol or nitrofurans in food and/or feed, and supported the data collection for the Comprehensive European Food Consumption Database, as well as the stakeholders that provided toxicity studies, usage levels of carrageenan (E 407), or information on semicarbazide in seaweeds.*

R8
AN INVESTIGATION INTO SOURCES OF CONTAMINATION OF CATTLE WITH THE VETERINARY DRUG PHENYLBUTAZONE

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Phenylbutazone (PBZ), also known as "bute", is a non-steroidal anti-inflammatory drug (NSAID) authorised to treat horses suffering from musculoskeletal disorders such as rheumatoid and arthritic diseases and to relieve them from the associated pain. An assessment of PBZ by The Committee for Medicinal Products for Veterinary Use (CVMP) (European Medicines Agency, 1997, EMEA/MRL/297/97-FINAL) found that the main health risks to the consumer were blood dyscrasias and the genotoxic/carcinogenic potential for which no thresholds could be identified and so no maximum residue limits could be established. As a consequence of this assessment PBZ is not permitted for use in any animal destined for the food chain. In addition to the EU, PBZ is banned from use in food production animals in most other countries including the USA, Canada and Japan. There is potential for unauthorised use of the drug in beef or dairy cattle and small numbers of bovines have tested positive for the drug in recent years as part of the European monitoring of veterinary medicinal product residues and other substances in live animals and animal products. Reports of The European Food Safety Authority indicate that 0.3% of the bovines tested in the EU from 2008 to 2012 were found to contain detectable concentrations of the drug. There is also anecdotal evidence that animals which have not been treated with the drug but have been present on a farm when another animal has been treated have also displayed levels of the drug. This study was designed to determine how and if contamination of bovines with PBZ could occur. The routes of contamination investigated were: i) from a feeding vessel previously used to administer the drug but not cleaned after doing so; ii) from close contact with a treated animal; iii) from pasture occupied by a treated anim al immediately before; iv) from pasture occupied by a treated animal three weeks before. The study was performed using PBZ treated bovines to act both as illegally treated cattle and as surrogates for legally treated horses in the scenarios described which could lead to contamination of other cattle.

Keywords: *phenylbutazone, veterinary drug, residues, contamination*

R9
MULTI-RESIDUE DETERMINATION OF ANTIBIOTICS IN EUROPEAN SEA BASS LIVER SAMPLES (DICENTRARCHUS LABRAX) THROUGH UHPLC–MS/MS

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The present work describes a method for the detection and quantification of antibiotics representative of seven classes, in European sea bass (*Dicentrarchus labrax*) liver tissues. Sulfonamides, trimethoprim, tetracyclines, macrolides, quinolones, penicillins and chloramphenicol were simultaneously determined through ultra-high-performance liquid chromatography with tandem mass spectrometry (UHPLC–MS/MS). Several procedures for sample treatment and extraction were tested to achieve the most suitable conditions. Validation was achieved following Decision 2002/657/ EC guidelines with precision, recovery, CCα and CCβ determined according to maximum residue limit (MRL) or the minimum required performance limit (MRPL), depending on compounds. With validation, the method was proven suitable for routine analyses in the detection and confirmation of antibiotics in liver of European sea bass, an economically important marine fish species, intensively reared in aquac ulture.

Keywords: *antibiotics, multi-residue, aquaculture, UHPLC–MS/MS, fish*

Acknowledgement: *This work was supported by FEDER through the Operational Program for Competitiveness Factors – COMPETE and by FCT – Portuguese Foundation for Science and Technology under the grant attributed to Sara Leston (SFRH/BPD/91828/2012) and through the project PTDC/AGR-ALI/122119/2010.*

R10
EVALUATION OF FIVE ANTIBIOTICS RESIDUES IN FARMED EUROPEAN SEA BASS (DICENTRARCHUS LABRAX) MUSCLE TISSUES THROUGH MEDICATED FEED

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Veterinary drugs are widely administered to guarantee and increase production efficiency by preventing and treating diseases and assure overall animal welfare in aquaculture. Among the pharmaceuticals most prescribed, five antibiotics (oxytetracycline, sulfadiazine, trimethoprim, oxolinic acid and flumequine) were selected to establish their residues retention in sea bass (*Dicentrarchus labrax*) muscle tissues. Fish were fed manually for 7 days with experimental diets containing two concentrations of each antibiotic, based on prophylactic and therapeutic dosages. After drug administration nine sampling times were established during the following 28 days, with muscle samples collected for antibiotics analyses through a validated multi-residue ultra-high-performance liquid chromatography with tandem mass spectrometry (UHPLC–MS/MS) quantification method. All the compounds surveyed were detected and quantified in sea bass and results suggesting a review on withdrawal times for some antibiotics analyzed.

Keywords: *aquaculture, antibiotics, fish muscle, UHPLC–MS/MS, drug residues*

Acknowledgement: *This work was supported by FEDER through the Operational Program for Competitiveness Factors – COMPETE and by FCT – Portuguese Foundation for Science and Technology under the grant attributed to Sara Leston (SFRH/BPD/91828/2012) and through the project PTDC/AGR-ALI/122119/2010.*

R11
ANIMAL DRUG RESIDUES…WHERE DO THEY RESIDE?

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Animal Drug Residues are a becoming more of a concern for many reasons. The FDA recently released the Veterinary Feed Directive (VFD) "to promote the judicious use of antimicrobials in food-producing animals". Veterinary drugs are used for a wide variety of reasons. Some of these include treatment of disease, herd health management, growth promotion, meat quality improvement and many other reasons. Several of these drug residues can remain in the animal and as a result enter into the food chain. A limited study was performed to determine where the drug residues were located and at what concentration. Samples of urine, blood, muscle tissue, liver and fat from each animal were obtained and tested by LC–MS/MS. Preparation and analysis of the samples was performed using a modified FSIS method. Data obtained surveyed 29 different antibiotics and growth promoters.

Keywords: *veterinary, antibiotics, hormones, drugs*

R12
HIGH-THROUGHPUT SALTING-OUT ASSISTED LIQUID–LIQUID EXTRACTION COUPLED WITH ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY METHOD FOR THE MONITORING OF TETRACYCLINES IN INFANT FOODS

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Tetracyclines (TCs) are broad-spectrum antibiotic agents that are used in animal husbandry for the control and treatment of bacterial infection. The presence of residues in milk or edible animal tissues could produce allergic reactions or dangerous effects on human health. In order to guarantee food safety, the European Union has established maximum residue limits (MRLs) of 100 µg/kg for TCs in foodstuff of animal origin, such as milk and meat muscle. However, there are not specific regulations in the case of infant foods. In this work a sensitive method based on salting-out assisted liquid–liquid extraction (SALLE) has been evaluated for the determination of residues of 5 common TCs in some infant foods such as infant yogurt, infant milk, follow-on milk, lacteal milk product and baby food, using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS). The separation of TCs was achieved in less than 4 min, using a Zorbax Eclipse plus RRHD C18 column (50 mm × 2.1 mm, 1.8 µm), with a mobile phase of water (0.01% formic acid) and methanol. The analytes were detected in ESI+ with multiple reactions monitoring mode. Parameters affecting the SALLE procedure such as the type and volume of the organic solvent, sample volume or type and amount of salt were studied. Under optimum conditions the extraction procedure was a follow: 1 mL of sample was treated with 1.5 mL of EDTA-McIlvaine buffer and vortexed. Subsequently, 3.2 mL of acetonitrile and 1.25 mg of NH_4SO_4 were added and the mixture was shaken and centrifuged. Then, the organic layer was transferred to a vial, dried under a N2 stream and the final residue was re-dissolved with 500 µL of H_2O:MeOH (95:5 *v/v*) and injected into the UHPLC–MS/MS system. Matrix effect was studied in different matrices being lower than $|21|$% in all cases, showing that the proposed procedure provides very clean extracts. Limits of quantification ranged from 0.16 to 0.48 µg/kg. The precision, expressed as relative standard deviations were below 10.5% in all studied matrices. Recoveries for fortified infant food samples ranged from 89.2 to 96.8%, with relative standard deviations lower than 7.3%. The results of the analysis revealed that the proposed SALLE–UHPLC–MS/MS method is simple, rapid, cheap and environmentally friendly, being successfully applicable for the determination of these residues in wide range of infant food products.

Keywords: *tetracyclines, infant foods, UHPLC–MS, SALTIng-out assisted liquid–liquid extraction*

Acknowledgement: *Excellence Project Ref: P12-AGR-1647. DMG thanks the Strengthening Program of I+D+i of the University of Granada for a postdoctoral contract.*

R13
PRODUCTION OF POLYCLONAL ANTIBODIES FOR THE DETERMINATION OF SEMICARBAZIDE IN MILK BY ENZYME-LINKED IMMUNOASSAY

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Nitrofurans, particularly Nitrofurazone (NFZ), belong to a class of synthetic broad spectrum antibiotics and mainly used for livestock, aquaculture and bee colonies in the prophylactic and therapeutic treatment of bacterial and protozoan infections. The use of nitrofurans has been prohibited completely in food animal production in the European Union (EU) since 1995 (Commission Regulation (EC) 1442/95) due to its carcinogenicity and mutagenicity; however, it is still widely used in Russia. The MRPL for nitrofuran metabolites residues is 1 μg kg^{-1}. NFZ metabolises rapidly after ingestion to form tissue bound metabolite - semicarbozide (SEM). Furthermore, metabolite residues are stable in tissues and persist for at least 6 weeks. As a result, effective analytical detection of these compounds could be carried out by the determination of bound metabolites. To avoid using labor-intensive instrumental methods to detect nitrofurans metabolite residues in food, a simple direct enzym e-linked immunosorbent assay (ELISA) method for nitrofurans determination was developed in this study. In this study, ELISA method was developed and optimized to allow the detection of the SEM residues in milk. A new adjuvant "Floravit" was used for the production of polyclonal antibodies against cp-SEM immunogen. "Floravit" is a bioactive additive based on cultural medium of Fusarium sambucinum. Three schemes of rabbit immunization were applied for anti-cp-SEM antibodies production:
1) Immunogen was emulsified with complete Freund's adjuvant for primary immunization; booster immunizations were performed using incomplete Freund's adjuvant and saline;
2) Immunogen was emulsified with complete Freund's adjuvant solved in 10^{-6} dilution of "Floravit" in saline for primary immunization, booster immunizations were performed using 10^{-6} dilution of "Floravit" in saline.
3) Immunogen was emulsified with complete Freund's adjuvant solved in 10^{-4} dilution of "Floravit" in saline for primary immunization, booster immunizations were performed using 10^{-4} dilution of "Floravit" in saline.
The antibody titer, the IC50 and the antibodies specificity were assessed by direct ELISA. The IC50s for np-SEM were (15.5±2.2); (9.7±4.6); (9.2±4.53) μgL^{-1} for antibodies produced by the 1st, 2nd and 3rd scheme of immunization subsequently. Cross-reactivity with other metabolites of Nitrofuran was less than 1%. It concluded that 10_{-4} dilution of "Floravit" in saline could be considered as a suitable for the production of polyclonal antibody to cpSEM immunogen. The antibodies produced by last scheme of immunization were applied for the determination of SEM in milk. The method was validated, in line with EU requirements (Commission Decision 2002/657/EC) concerning screening methods. Recoveries higher than 76% were obtained. The limit of detection was 0,4 μg kg^{-1} for semicarbazide.

Keywords: *semicarbazide, polyclonal antibody, ELISA*

Acknowledgement: *We thank to LLC "Gella-Pharma" (Moscow, Russian Federation) for bioactive additive "Floravit"*

R14
CAPILLARY ELECTROPHORESIS AND CAPILLARY ELECTROCHROMATOGRAPHY AS ALTERNATIVES FOR 5-NITROIMIDAZOLE RESIDUE DETERMINATION IN MILK SAMPLES

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5-nitroimidazoles (5-NDZs) are human antimicrobials with a wide action spectrum, being very effective against anaerobic protozoan and bacteria. However, their use as veterinary drugs in animals intended to human consumption is forbidden in European Union (EU) because of several studies have attributed carcinogenic, genotoxic and mutagenic properties to these compounds. Although 5-NDZ residues are not allowed in food of animal origin, the Rapid System of Food and Feed (RASFF) portal is still registering alerts about their presence in food matrices. Traditionally, 5-NDZ determination has been carried out by liquid chromatography (LC) while the use of capillary electrophoresis (CE) or capillary electrochromatography (CEC) as analytical tools has been barely explored for this purpose. In this work, two new efficient alternatives based on CE and CEC are presented for 5-NDZ residue monitoring in milk samples. A micellar electrokinetic capillary chromatography (MEKC) method coupled to UV detection has been developed for the determination of nine 5-NDZs in less than 18 min. Prior to sample injection, milk fat and proteins were simultaneously removed by the addition of trichloroacetic acid (TCA) and subsequent centrifugation. Afterwards, sample clean-up and off-line concentration were achieved by a solid-phase extraction (SPE) procedure employing Oasis® MCX cartridges. Analyses were performed in a fused silica capillary (61.5 cm × 50 μm i.d.) with an optical path length of 150 μm, employing 20 mM phosphate buffer (pH 6.5) and 150 mM SDS as background electrolyte (BGE). During the separation procedure, a temperature of 20°C and a voltage of 25 kV (normal mode) were applied. Due to sweeping effects, an on-line concentration was achieved for all the studied compounds and detection limits lower than 1.8 μg/L were obtained. Method characterization resulted satisfactory in terms of li nearity (R^2≥0.990), repeatability (≤14.3%) and reproducibility (≤20.1%). Another proposal is the determination of eight 5-NDZs by CEC-UV, carried out in laboratory packed capillaries in less than 15 min. Separation was performed in a C18 packed capillary (40 cm effective length and 50 μm internal diameter) using a mixture 60:40 acetonitrile:buffer (ammonic acetate, pH 5, 1 mM) as liquid mobile phase. Separation took place at 30°C under an applied voltage of 27 kV. Milk samples were pretreated by salting out assisted liquid-liquid extraction (SALLE) followed by a SPE employing Oasis®HLB cartridges. After, they were hydrodynamically injected for 120 s at 11.5 bars. Method characterization resulted satisfactory in terms of linearity (R^2 ≥ 0.992), repeatability (≤ 12.2%) and reproducibility (≤ 14.5%), obtaining detection limits lower than 28.8 μg/L for all studied compounds.

Keywords: *5-nitroimidazoles, capillary electrophoresis, capillary electrochromatography, milk samples.*

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R15
DETERMINATION OF 5-NITROIMIDAZOLE ANTIBIOTICS IN MILK SAMPLES BY UHPLC–UV COUPLED WITH SALTING-OUT ASSISTED LIQUID–LIQUID EXTRACTION AS SAMPLE TREATMENT

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In the recent years the interest on Green Analytical Chemistry has involved a great development of sample treatment procedures. In this sense, salting-out assisted liquid-liquid extraction (SALLE) has proved to be an effective sample treatment, showing as main advantages the simplicity in operation, low cost, reduction the extraction time and use of non-halogenated or aromatic solvents compared to the conventional liquid-liquid extraction. In this work, an exhaustive study of parameters involved in a SALLE procedure has been carried out for the extraction of eight 5-nitroimidazole (5-NDZ) antibiotics from milk samples, previous to their analysis by ultra-high performance liquid chromatography (UHPLC) with UV detection. From the optimization study, ethyl acetate (EtAc) and sodium sulphate (Na_2SO_4) were established as extraction solvent and salting-out agent, respectively. The influence on the extraction efficiency of different variables involved in the SALLE, such as salt amount, EtAc volume and agitation and centrifugation times, was evaluated through a screening experimental design. Finally, optimum values of these parameters were subsequently obtained through a surface response Doehlert design. The final SALLE procedure consisted on the following steps: 4 mL of milk sample were centrifuged for 10 min at 7,500 rpm and most fat was removed. Afterwards, 10 mL of EtAc were added to the supernatant and the mixture was centrifuged for 5 min at 7500 rpm. Then, 1.0 g of Na_2SO_4 was added to the sample followed by centrifuging for 10 min at 7500 rpm. Finally, 6.3 mL of the organic supernatant were recovered. This volume was dried under nitrogen current at 40°C and it was reconstituted in 200 µL of acetonitrile (MeCN):water 6:94 (*v/v*) containing formic acid 0.1% (*v/v*). This extract was filtered and analysed by UHPLC, using a C18 Zorbax Eclipse Plus column (50 mm x 2.1 mm, 1.8 µm) in gradient mode, using 0.1% (*v/v*) formic acid aqueous solution and acetonitrile containing 0.1% formic acid (*v/v*) as mobile phase solvents. Separation was performed in 8 min under a mobile phase flow rate of 0.45 mL/min. Column was thermostatically controlled at 45 °C using an injection volume of 20 µL. Analytical signals were monitored at 320 nm. Matrix-matched calibration curves showed satisfactory linearity ($R^2 \geq 0.996$). Detection limits ranging from 2 to 4 µg/L were obtained. Precision studies resulted in relative standard deviations (RSDs) lower than 10% and recoveries over 71.1% were obtained for all studied compounds in milk samples.

Keywords: *5-nitroimidazoles, salting-out assisted liquid-liquid extraction (SALLE), milk samples, ultra-high performance liquid chromatography (UHPLC)*

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R16
A RAPID SCREENING ASSAY FOR THE SIMULTANEOUS DETECTION OF ANTIMICROBIAL AGENTS IN BOVINE MILK BY LIQUID CHROMATOGRAPHY COUPLED WITH AN ACCESSIBLE MASS DETECTOR

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An important aspect relating to the use of veterinary medicines in cattle is the presence of residues in milk. Antimicrobials constitute the largest class of compounds administered to livestock globally. This widespread use together with stringent food safety legislation necessitates the availability of rapid and sensitive analytical techniques for residue detection. Cost-effective, robust and broad-scope platforms, which can be easily implemented in routine control laboratories are of importance. Other considerations are the flexibility of analytical scope and the extent of compliance with internationally recognised validation criteria. The screening analysis of antimicrobial residues, as required by European Union (EU) Regulation 2002/657/EC will be discussed on a compact single quadrupole mass detector. A simple dispersive solid phase extraction (d-SPE) procedure provided effective and simple sample clean up, where average recoveries were > 90% for all multi class analytes. Liquid chromatography separation, coupled with an accessible single quadrupole mass detector provided robust analysis and high sample throughout, where the screening target concentrations (STC) were less than the EU MRLs for each analyte. The method was found to be fit for purpose, thus allowing for reliable and rapid screening of commonly administered multi residue antibiotics below EU regulatory limits in bovine milk.

Keywords: *veterinary drug residues, milk, QDa, screening*

R17
A NEW SORBENT FOR CLEANUP OF SEAFOOD EXTRACTS PRIOR TO MULTIRESIDUE VETERINARY DRUG LC–MS ANALYSIS

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Seafood and other tissue samples are typically extracted with an acetonitrile based solvent for LC–MS determination of veterinary drug residues. Among the most significant co-extractable substances are fats and polar lipids, particularly phospholipids. For example, a 1 gram sample of shrimp muscle typically contains about 100 mg of fat and about 5 mg of phospholipids. Reversed-phase sorbents such as C18 are effective for removal of fat from the acetonitrile based extraction solvent, but are ineffective for removal of phospholipids. Excessive amounts of phospholipids can shorten LC column life and contribute to ion-suppression and contamination in the mass-spectrometer. Results indicate that this new sorbent is highly effective for removal of both phospholipids and fats from meat and milk extracts prior to LC–MS analysis. With the new sorbent, recoveries of veterinary drugs were similar to results obtained using C18 for cleanup, but phospholipid removal was greater than 80% better.

Keywords: *veterinary drug residues, sample cleanup, Oasis PRiME HLB, LC–MS/MS*

R18
THE UTILITY OF ION MOBILITY IN AN ACCURATE MASS SCREENING WORKFLOW FOR THE DETECTION OF VETERINARY DRUG RESIDUES IN COMPLEX MATRICES

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Full spectral acquisition, high mass resolving power and accurate mass measurements are accepted as essential attributes for successful multi-residue screening analysis. Nonetheless, correctly assigning the identity to detected residues in the presence of isobaric interference, from matrix-derived co-extractive components, still remains a serious challenge. Sometimes mass resolution and chromatographic separation are not enough. Ion mobility utilises an additional dimension of separation, based on molecular size and shape, which can separate isomers, remove isobaric/matrix interferences, generate cleaner mass spectra and calculates collision cross section (CCS) values to aid identification. Here we evaluated the performance of UPLC coupled to ion mobility enabled QTOF mass spectrometry for the comprehensive screening of residues of 90 veterinary drugs, belonging to 11 classes, in a variety of matrices. Matrix-matched standards and extracts of bovine liver, urine and porcine muscle containing undisclosed concentrations of veterinary drug residues, generated for an inter-laboratory validation study [1], were analysed. UPLC separation was performed on a BEH C18 (1.7 µm, 2.1×100 mm) analytical column. Data acquisition was performed using a Synapt G2-Si HDMS mass spectrometer using electrospray in positive ion mode with ion mobility enabled non-selective precursor ion fragmentation (HDMSE). Data processing was performed using UNIFI (v.1.8) software. UPLC HDMSE data was acquired for solvent standard mixtures and was used to generate mobility separated MSE spectra. The precursor ion, two fragment ions, mobility drift time and values for CCS were determined for the 90 compounds and entered into the UNIFI scientific library. The measured CCS values for target compounds present in solvent standards, matrix-matched standards and extracts from validation samples were shown to be in good agreement (≤2% error). Results from acquisition using travelling wave and drift cell ion mobility cells were also consistent. Ion mobility drift times are independent of matrix and can be utilised as an additional parameter to reduce false detects, avoid false negatives and improve confidence in non-targeted screening.

[1] Performance criteria for multi-veterinary drug-residue analytical methods; Joint Defra VMD and industry sponsored project (project number DEFRA R3; RIKILT 1237312301)

Keywords: *ion mobility, screening, collision cross section, veterinary drug residues*

Acknowledgement: *Defra/VMD funding (VMO512)*

R19
BEADYPLEX: A NOVEL MULTI-ANTIBIOTIC FLOW CYTOMETRIC SCREENING METHOD FOR FOOD COMMODITIES

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Misuse of antibiotics with therapeutic, prophylactic or growth promoting purposes may result in the presence of residues in edible animal products, a potential threat to consumer health. In addition to allergic or toxic adverse effects reported for some antimicrobials, the extensive worldwide abuse of antibiotics has been correlated to the increase of pathogen resistance development observed in the recent years, a growing global concern for both human and veterinary medicine. Furthermore, the presence of antibiotic residues in raw materials may hamper fermentation processes (e.g. in dairy or meat derivative products), generating important economic losses for the food industry.
Consequently, efficient screening analytical methods for the early detection of antibiotic residues are essential to guarantee food safety and consumer protection. The current tendency in food analysis entails the implementation of multi-residue technologies which considerably improve analysis time and global cost. Flow Cytometric Immunoassays (FCIAs) combine the detection of receptor-ligand interaction events by immunoanalysis with the multi-parametric characterization of microparticles by Flow Cytometry. This feature provides a great multiplexing capability, making FCIAs especially suitable analytical tools for the simultaneous monitoring of multiple targets. Other remarkably advantages are high-throughput capability, high sensitivity and specificity and in some extent simplicity, rapidity, cost-effectiveness. Herein we present BEADYPLEX, a new competitive FCIA that allows the analysis of more than 80 antimicrobial residues from 10 families widely used in the veterinary field, including tetracyclines, sulfonamides, β-lactams, aminoglycosides, macrolides, fluoroquinolones, lincosamides, phenicoles, polymyxines and pleuromutilins. Beadyplex has been developed using receptors/antibodies covering most relevant antimicrobial families and individually encoded microparticles covalently coupled to different antibiotic competitor molecules. This test combines 12 immunoassays in the same single reaction, which in addition to multi-antibiotic determination, provides the identification of residues at the family level in one unique analysis per sample. A rapid and solvent-free extraction method has been developed for the universal application of Beadyplex to different food matrices, including tissue (porcine, poultry, bovine muscle), eggs, fish (salmon and tuna), seafood and raw milk. This user-friendly procedure requires few equipment and allows the analysis of hundreds of samples per day. Antibiotics included in the scope of the method are detected at concentrations relevant to industries and European authorities (at or below regulatory limits for most residues). The method has been validated by the Belgian National Reference Laboratory CER Groupe for porcine muscle following the European CRL guidelines for screening methods. This study confirms the suitability of using Beadyplex as screening method for the multi-residue monitoring of antibiotics in food commodities.

Keywords: *flow cytometry immunoassay, antibiotics, multi-residue screening, multi-matrix, beadyplex*

Acknowledgement: *This work fits into the Eurostars project Go-With-The-Flow E6993 and has received funding from Eureka, European community and Belgian Walloon Region.*

R20
TARGETED MULTI RESIDUE LC–MS/MS METHOD FOR SULFONAMIDES AND NITROIMIDAZOLES ANTIBIOTICS IN HONEY WITH LC–MS/MS

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Bee products are used for its nutritional and medicinal value. Health safety of honey products is primary concern in many countries including America and European Union. Farmers extensively use various antibiotics and other chemicals to treat diseases in bees. To control the usage of antibiotics, stringent regulations are laid down globally for permitted levels in honey. A great demand exists for short and reliable multi-residue methods that detect and quantitate maximum number of analytes in a single run. Fast and robust quantitation method is developed to identify and quantify antibiotics from two different antibiotic classes in honey samples covering 13 different analytes. Raw and processed honey can be screened and quantified for the antibiotics with the proposed validated method. Multiple Reaction Monitoring (MRM) method developed on the 4500 QTRAP® system. Two MRM transitions per analyte were selected. Analysis performed on Shimadzu HPLC on reverse phase C18 phase with gradient elution. Mobile phase of Water with Acetonitrile and methanol mixture containing formic acid was found effective for separation. Flow rate, injection volume and column conditions were optimized. Data processing and quantitation was done on Analyst software with Multi Quant™ quantitation software. For standard calibration curve preparation, blank matrix was fortified with antibiotic mix working standards. Spiked samples extracted with methanol/water, vortexed, sonicated and centrifuged. Supernatant, filtered and aliquots injected. The unknown honey samples from local market were subjected to the same sample preparation protocol for detection and quantitation. Method validation performed as per EU Commission Decision 2002/657/EC guidelines. Parameters of Sensitivity, Linearity, Accuracy, Precision, Limit of detection (LOD) and quantitation (LOQ) were established. Good sensitivity of method is highlighted by the Limit of quantitation (LOQ) achieved at 0.5 µg/kg for Sulfonamides and 0.2 µg/kg for Nitroimidazoles respectively. Linear Dynamic Range (LDR) of 3 orders and more observed for all analytes. Calibration curves obtained were linear from 0.5 µg/kg to 500 µg/kg for sulfonamides and 0.2 to 500 µg/kg for Nitroimidazoles satisfying the EU requirement. The regression coefficients of r > 0.99 and above were obtained. Recovery and reproducibility investigated by spiking blank honey matrix at concentration of 0.5 µg/kg for Sulfonamides and 0.2 µg/kg for Nitroimidazoles antibiotics. Accuracy range for all analytes found between 78 to 115%. Method shows good precision data with acceptable % CV range from 3% to 17% for different analytes.

Keywords: *sulfonamides, nitroimidazoles, honey, LC–MS/MS*

R21
PRODUCTION OF ANTISERA TO PHENYLBUTAZONE AND OXYPHENYLBUTAZONE FOR USE IN IMMUNOCHEMICAL DETECTION ASSAYS

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Phenylbutazone is a non-steroidal anti-inflammatory drug that is commonly used to treat horses for the pain associated with musculoskeletal disorders. However the drug is known to cause adverse effects in humans and is therefore not permitted for use in any animal destined for human consumption. In compliance with European legislation, samples from food producing animals are analysed for the presence of the drug and in recent years some equine and bovine samples have proved to be non-compliant. While physicochemical methods of analysis exist there is a need for less expensive, rapid screening methods such as immunoassays. This study describes the production of antisera that could be employed in an immunoassay to detect the two marker residues, phenylbutazone and its main metabolite, oxyphenylbutazone. Four haptens were chosen for immunogen preparation and two of these, suxibuzone and γ-hydroxyphenylbutazone, produced antisera which had IC_{50}s of < 5 ng mL^{-1} for phenylbutazone. Antisera with IC_{50}s of 5.5 ng mL^{-1} for phenylbutazone and 5.6 ng mL^{-1} for oxyphenylbutazone were produced by the phenylbutazone and oxyphenylbutazone haptens respectively. These figures suggest that when incorporated in immunoassays the antisera could deliver detection capabilities below the concentration that is recommended by the Community Reference Laboratory (5 ng mL^{-1}).

Keywords: *residues, phenylbutazone, oxyphenylbutazone, antisera, immunoassay*

R22
MULTIRESIDUE DETERMINATION OF 17 BETA-AGONISTS IN BOVINE HAIR USING QUICK AND EASY EXTRACTION AS A CLEANUP IN COMBINATION WITH ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (UHPLC–MS/MS)

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The non-therapeutic use of β-agonists as growth promoters in livestock is banned by the EU. In order to control the potential misuse and to adhere to the global regulatory requirements, it is vitally important to monitor the presence of β-agonists in different matrices. The bioaccumulation of drugs in hair turns this unconventional matrix in a powerful tool for monitoring of illegal growth-promoting treatments. The conventional methods for analysis of β-agonists in hair involve the use of prolonged hydrolysis, followed by SPE prior to the LC–MS/MS analysis. This makes these methods laborious, time-consuming and expensive. In the monitoring programmes on a routine basis, any labor, time and cost savings are beneficial. Thus, the objective of this work was to develop a quick, easy and cheap method for the analysis of β-agonists in hair that can allow cost-effective sample preparation and analysis with LC–MS/MS for quantitative confirmation of β-agonists . To this aim, the digestion of hair samples in NaOH solution was shortened, and the conventional SPE was replaced by a simple and quick liquid-liquid extraction. The analysis was carried out by UHPLC coupled to a triple quadrupole mass spectrometer. Despite the generic sample clean-up, good repeatability and reproducibility of both retention times and relative abundances of diagnostic transitions, allowed the successful identification of all analytes i.e salbutamol, terbutaline, zilpaterol, cimaterol, cimbuterol, fenoterol, clencyclohexerol, ractopamine, clenproperol, tulobuterol, clenbuterol, brombuterol, isoxsuprine, clenpenterol, mabuterol, mapenterol, salmeterol. The method was validated according to the Decision 2002/657/CE. The trueness ranged from 95.9% to 112.6%, with CCα from 0.5 µg/kg to 2 µg/kg. The repeatability varied between 2.2% to 9.1%, while the intra-laboratory reproducibility (CV%) was between 4.0% and 23.7%. The method is suitable for quantification a nd confirmation of β-agonists except for fenoterol.

Keywords: *beta-agonists, hair sample preparation, UHPLC–MS/MS, multiresidue analysis, clenbuterol*

R23
DETERMINATION OF STEROID HORMONES IN MUSCLE USING QUECHERS SAMPLE PREPARATION APPROACH AND QTRAP® LC–MS/MS

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Liquid chromatography tandem mass spectrometry analytical method for the determination of fifteen steroid hormones from the group of androgens, estrogens and acetyl-gestagens in animal muscle has been developed and validated. Sample preparation utilized the QuEChERS method with ethyl acetate as extraction solvent followed by sample clean-up by dispersive solid phase extraction with C18, primary secondary amine sorbent and magnesium sulphate. For chromatographic separation of steroid hormones, a Poroshell 120-EC C18 analytical column (150 × 2.1 mm, 2.7 µm) allows to get good selectivity and peak shapes was used. The mobile phases consisting of methanol/water mixture (70:30, *v/v*) for androgens and estrogens as well as acetonitrile/methanol/water mixture (20:50:30, *v/v/v*) for acetyl-gestagens were pumped in isocratic modes at the total flow rates of 150 µL min-1 and 200 µL min-1 respectively. The column was maintained at a constant temperature of 45°C. The analytes were determined and identified by liquid chromatography tandem mass spectrometry on QTRAP 5500 instrument equipped with a TurboIon-Spray source operating in positive and negative electrospray ionization modes and controlled by Analyst software. Depending on the target steroid hormone at least two transitions in the multiple reactions monitoring mode were monitored. The method was validated in accordance with the Commission Decision 2002/657/EC performance criteria. The results of achieved apparent recoveries of all examined compounds were in the range from 60.9 to 133.0%. The reapetability was less than 25% and reproducibility did not exceed the limit of 30%. For all hormones studied the regression parameters of linearity were correct, with a correlation coefficients greater than 0.98 indicated a good fit, in the whole range of tested concentrations. The decision limits (CCα) ranged from 0.04 µg kg^{-1} for megestrol acetate to 0.34 µg kg^{-1} for 17β-19nortestosterone, while the detection capabilities (CCβ) ranged from 0.07 µg kg^{-1} to 0.57 µg kg^{-1} respectively. All the obtained CCα and CCβ values were below recommended concentration - 1 µg kg^{-1} for the steroid hormones tested. The analytical method meets the criteria for confirmatory methods and will be used in the official control of hormones.

Keywords: *steroid hormones, liquid chromatography, mass spectrometry*

R24
WHICH MARKER RESIDUES FOR CARBADOX IN PIG TISSUES? - VALIDATION OF A LC–MS/MS METHOD FOR THE DETERMINATION OF METABOLITES OF CARBADOX IN MUSCLE AND LIVER.

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Carbadox, an antibacterial agent, is a quinoxaline NN dioxide compound that has been used in the past as feed additive for piglets in veterinary practice. It was used as an antimicrobial growth promotant and for the prevention and treatment of enteric diseases. It is rapidly metabolized into N-O reduced compounds as mono-desoxycarbadox, di-desoxycarbadox (DCBX) and quinoxaline carboxalic acid (QCA). As CBX and DCBX are suspected to be carcinogens and mutagens, the use of carbadox has been banned in European Union since January the 1st, 1999 to protect the health of consumers, animals and industrial manipulators in the feed production chain. However these compounds are still authorized as a veterinary product in other countries like Canada. In 1991, to control misuse of these compounds, JECFA designated QCA (quinoxaline carboxylic acid) as marker residues for carbadox in liver as target tissue. However, from recent kinetic studies, Canada argues that the marker residue for carbadox should be preferentially DCBX in replacement of the QCA. So, in this context, to answer to the question: “which marker residues for carbadox in pig tissue?” a depletion study was undertaken at our experimental premises to measure the depletion of the two metabolites QCA and DCBX in muscle and in liver. Pigs were fed with carbadox, and tissues were collected and measured for the QCA and DCBX content using a LC–MS/MS method. This poster presents the performance criteria of the LC–MS/MS analytical method developed and validated for this purpose. From measurement, QCA and DCBX muscle and liver profiles after administration of carbadox are presented and allow predicting which compound is more persistent in animal tissues.

Keywords: *carbadox, residue, control, pig, muscle*

R25
IMPLEMENTATION OF AN OPTIMAL ANTIBIOTICS RESIDUES CONTROL PLAN IN IMPORTED AQUATIC PRODUCTS BY ELISA TESTING

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The IFS and BRC referentials establish a decisive framework for food quality management systems set up by food companies. Their latest releases include reinforcing the requirements, particularly on purchases. The internal monitoring plan, developed on the basis of identified potential contaminants, is one of risk management elements, required for the quality approach. The autocontrol plan HACCP is also an obligation for food companies that will be held responsible for the marketing of a non-compliant product. The European Regulation 37/2010 establishes an MRL for authorized substances, and the minimum performance required MRPL for those constituting an hazard for human health. The Council Directive 96/23 EC lays down measures to monitor these substances and group of residues. Implementation of national residues monitoring plans in the member states 2010, 2011 and 2012 have shown the Dyes, first of all the Malachite Green followed by Cristal Violet, are far the most frequently residues found among all antibiotics: Mean of 38/41 By compiling nonconformities caused by antibiotic residues identified in the European RASFF portal from 01/2014 to 03/2015 (70 within 15 months) it appears clearly that only few sustances are found 93 % of the cases are linked to 5 molecules or groups: For fish and fish products: Nitrofuran SEM and Malachite green For shrimps and shellfish: Tetracyclines, Chloramphenicol and Nitrofuran AOZ More than 90 % of the risks on imported aquatic products could be covered by focusing on a control plan of those 2 or 3 antibiotics or family, specifically established for each matrice ELISA tests based onto the specific recognition of a molecule or a family can detect the presence of these compounds at a very low limit of detection for fish or crustacean and derivative matrices : -Nitrofuran SEM: LOD 0.2 µg/kg – MRPL set at 1 – Nitrofuran AOZ: LOD 0.1 µg/kg – MRPL set at 1 – Chloramphenicol: LOD 0.005 µg/kg – MRPL set at 0.3 – Tetracyclines: Detection of group & 4-epimers LOD 2 µg/kg – MRL set at 100 – Malachite green et Leucomalachite : LOD 0.25 µg/kg – MRPL set at 2 Specific protocols are available for easy implementation in laboratory with a result in half day or full day. The ease of use and ability to respond to the antibiotic risk meet all the timely decision requirements, essential for industrial or products importer which often cannot extend the storage. An upstream monitoring plan antibiotic risk, targeting key molecules by 2 or 3 ELISA tests can be set up with a mastered budget. It allow the manager to minimize the nonconformity product making in order to ensure high quality level product to its customers.

Keywords: *antibiotic, ELISA, fish, schrimps, Malachite green*

R26
SCREENING AND QUANTITATION OF VETERINARY DRUGS IN FOOD OF ANIMAL ORIGIN BY HIGH SENSITIVE TRIPLE TOF SYSTEM

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Vet Drugs in food are of growing concern in food safety. In order to properly assess the effects of such compounds in our food, it is necessary to accurately monitor their presence, but this provides a challenge with the ever increasing numbers and classes of compounds being evaluated. To aid in the analytical tests on such samples, Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS) has become a preferred tool for the analysis of a wide range of environmental pollutants. For targeted analysis, triple quadrupole mass spectrometry instruments have typically been used due to their inherent specificity and sensitivity. Using a targeted approach, however, means that you may be missing some of those important compounds, which will often mean the requirement of a secondary analysis. To overcome this, the advancements in LC–MS/MS technology, including hybrid systems like quadrupole-quadrupole Time-of-Flight (TripleTOF®), now provide the ability to perform qualitative and quantitative screening on a routine basis. TripleTOF® technology combines the best attributes of triple quadrupole and accurate mass TOF analyzers in a single instrument allowing quantitation with triple quadrupole-like performance (sensitivity, accuracy, linearity) and at the same time high confidence identification based on accurate mass MS and MS/MS information. The application of specific and sensitive mass spectrometers enables the development of fast and robust methods for the screening of veterinary drugs residues. Good sensitivity of method is highlighted by the Limit of quantitation (LOQ) achieved low ppt level. Linear Dynamic Range (LDR) of 4.5 orders and more observed for analytes. Method also shows good precision data with acceptable %CV range below 10% for different analytes. Furthermore, Information Dependent Acquisition (IDA) and SWATH are demonstrated as very powerful tools for confirming the screening results.

Keywords: *high resolution system, veterinary drugs, quantitation, IDA, SWATH*

R27
IDENTIFICATION AND DETERMINATION OF 19 B-AGONISTS IN FOOD PRODUCTS AND FEEDS BY LC–ESI–QTOF

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Growth promoters - β-agonists have an impact on the productivity of animals and poultry, help to increase muscle mass and have a significant fat burning effect that produces lean meat. The use of growth promoters in the breeding of livestock and poultry in the European Union, China and Russia is prohibited under the strict supervision of veterinary authorities, but in some other countries (United States, Canada, Argentina, etc.), some β-agonists are still applicable. Nowadays, the identification and determination of residual amounts of β-agonists in food and feed is carried out mainly by high performers liquid and gas chromatography tandem mass spectrometry (HPLC/GC–MS–MS). Extraction of β-agonists carried by buffers after enzymatic or acidic hydrolysis, followed by solid phase extraction. Disadvantages of existing methods of sample preparation are duration (hydrolysis is carried out for several hours) and multistage cleaning operation. The method for the simultaneous identification and determination of 19 β-agonists (brombuterol, hydroxymethyl clenbuterol, zilpaterol, isoxsuprine, carazolol, clenbuterol, clenpenterol, clenproperol, xylazine, mabuterol, mapenterol, ractopamine, salbutamol, salmeterol, tulobuterol, fenoterol, formoterol, cimaterol, cimbuterol) in animal feed, meat, liver and kidneys of animals by high-performance liquid chromatography with time of flight mass spectrometry, and simple and fast sample preparation have been proposed. Matrix effect under electrospray ionization eliminated by diluting the extract 10 – fold amount of deionized water. A scheme for the identification and determination of contaminants by method of standard addition was proposed. Limit of Detection CCα - 0.1–7 ng/g, detection capability CCβ – 0.3–8 ng/g. The relative standard deviation of the results does not exceed 0.13. The identification period was 40–60 min, and the determination of identified toxicants was in the range of 2–3 hours. The duration of the analysis of 1–2 hours.

Keywords: *beta-agonists, feed, food products, HPLC, time-of-flight mass spectrometry*

R28
RAPID ANALYSIS OF SEDATIVES, BASIC AND ACIDIC NSAID´S IN KIDNEY BY LC/MS/MS

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A rapid method for quantitative and confirmative analysis of sedatives, basic and acidic NSAID´s (non steroidal anti-inflammatory drugs) in kidney using liquid chromatography-tandem mass spectrometry (LC/MS/MS) was developed and validated. The method includes acepromazine, 2-(1-hydroxyetyl) promazine sulfoxide (HEPS), propionylpronazine, xylazine, azaperone, azaperol, carazolol, 5-hydroxyflunixin, flunixin, meloxicam, diclofenac, phenylbutazone, oxyphenbutazone, ibuprofen, ketoprofen, vedaprofen, carprofen, naproxen, tolfenamic acid, mefenamic acid, firocoxib, rofecoxib, celecoxib and four metamizole-metabolites. The kidney samples were shaked with ammonium acetate, water and acetonitrile followed by centrifugation. A part of the supernatant was diluted and analysed by LC/MS/MS (Waters Xevo TQS), another part of the supernatant was evaporated to dryness and then reconstructed before analys. The analytes were separated on a reversed phase column (Waters ACQUITY) and detected by electrospray ionization followed by multiple reaction monitoring (MRM). The validation was performed according to the Commission Decision 2002/657/EC at 10-100 µg/kg for the sedatives, 100 µg/kg for the basic NSAID´s and at 5–1,000 µg/kg for the acidic NSAID´s. For unauthorised NSAID´s the target value was set to 5 µg/kg and for unauthorised sedatives the target value was set to 50 µg/kg. The within-laboratory reproducibility was below 20% and trueness was within ±20% for almost all compounds.

Keywords: *non steroidal anti-inflammatory drugs, sedatives, residue analysis, kidney*

R29
INVESTIGATING A POSSIBLE SOURCE FOR CHLORAMPHENICOL CONTAMINATION OF SWEDISH HONEY

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Chloramphenicol (CAP) is an antibiotic with some serious side effects, such as aplastic anemia, a rare but fatal blood disorder. Therefore, CAP is listed as a prohibited substance for use in food producing animals with a proposed Reference Point for Action (RPA) of 0.3 µg/kg. The National Food Agency in Sweden has a monitoring program of CAP in honey, and during 2011–2014 CAP residues (in avg. 0,07 µg/kg) were found in samples from four different beekeepers. An investigation was made, and no illegal or intentional use of CAP could be proven. Therefore it was hypothesized that imported beeswax (perhaps not intended for food production) could be the source. For the analysis of beeswax, an LC–MS/MS based method with a rapid sample preparation step was developed and validated. The method performed well and fulfilled the criteria laid down in Commission decision 2002/657/EC, with a CCα of 0.07 µg/kg and a CCβ of 0.12 µg/kg. CAP was confirmed in ten samples of a type of beeswax that may have been used as a foundation for beehive colonies, and the levels ranged between 0.29–0.58 µg/kg. Given that CAP was not applied intentionally by the Swedish beekeepers, the use of contaminated beeswax is a plausible source of the CAP residues found in Swedish honey.

Keywords: *chloramphenicol, residues, honey, beeswax, LC–MS/MS*

R30
DEVELOPMENT AND VALIDATION OF A QUANTITATIVE METHOD FOR B-AGONISTS IN PIGS AND CATTLE MUSCLE BY LC–MS/MS

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The use of β-agonists as growth promoters in pigs and cattle is banned in several countries due to its potential risk to human health. Seeking to increase the food security of the population and in order to attend the PNCRC – Brazilian Residues and Contaminants Control Plan a method for simultaneous determination of five beta-agonists (cimaterol, clenbuterol, ractopamine, salbutamol and zilpaterol) in muscle was developed and fully validated including levels below 1 µg/kg. The sample extraction was conducted by a liquid/liquid extraction, of the homogeneized muscle, using methanol followed by an addition of hexane to eliminate residual fat in the meat. The extract was enzymatically hydrolyzed using β-glucuronidase overnight and the clean-up was carried out using class-selective MIP cartridges. Chromatographic separation was performed by HPLC (1200 Agilent) using Supelco C18 column (5 cm × 2.1 mm, 2.7 µm) with a mobile phase consisting of 0.1% formic acid in ammonium acetate 5 mM and methanol: 0.1% formic acid in ammonium acetate 5 mM (95:5) at a flow rate of 0.3 mL/min over a run time of 9 minutes. The mass analysis was performed in a triple quadrupole mass spectrometer (API 5000 – Sciex) with an electrospray ionization source in the positive ion mode. The compounds of interest were monitored using MRM where two fragments for each protonated molecule of analyte were selected. Level of interest was established for each compound being 5.0 µg/kg for zilpaterol, salbutamol and cimaterol in cattle and pigs; 0.1 µg/kg for clenbuterol and ractopamine in pigs; 0.2 µg/kg and 10 µg/kg for clenbuterol and ractopamine in cattle respectively. The validation comprehended essays for the evaluation of specificity, calibration curves, repeatability, within-laboratory reproducibility and ruggedness that was conducted following the criteria presented by decision 2002/657/EC. Decision limits and detection capabilities were determined. Value s for CCα ranged from 0.02 to 2.24 µg/kg and from 0.04 to 3.85 µg/kg for CCβ.

Keywords: *beta-agonist, validation, veterinary drug residues*

Acknowledgement: *This research was supported by CNPq and Ministry of Agriculture, Livestock and Food Supply – MAPA*

R31
EXPLOITING ANTIBODY AND RECEPTOR CROSS-REACTIVITIES TO DEVELOP BROAD RANGE MASTER-CURVE CALIBRATED ASSAYS FOR TETRACYCLINES AND SULFONAMIDES

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ELISA test kits are usually designed to be highly specific, with little or no cross-reactivity for substances other than the target analyte. On the other hand, during the official controls of residues in food and feed such high specificity is a considerable drawback preventing multiresidue detection of contaminants. Therefore, Tecna has recently done a big effort in the opposite sense developing ELISA kits for tetracyclines and sulfonamides with a broad range cross-reactivity pattern. As result, these ELISA tests allow to detect a whole class of structurally related molecules. Cross-reactivities were determined for seven tetracyclines and fifteen sulfonamides, by calculating the ratio between analyte concentration giving 50% signal inhibition to the cross-reactant concentration giving the same inhibition. Moreover, considering that the cross-reactivity of a binding assay could vary in matrix along the whole measuring range, cross-reactants spiked and naturally contaminated samples were analysed to verify the real suitability of the assay to detect all the molecules. These B ZERO kit formats for tetracyclines and sulphonamides, B ZERO TETRA HS and B ZERO SULFA, are a new generation of ELISA kits, with no need to run any calibrator but the "zero" standard. The removal of calibrators allows analysts to save wells and to exploit the whole kit for the analysis of routine samples. Besides, mistakes in calibration preparation and standard degradation are avoided since the calibration is provided as virtual batch-related master-curve. Both kits have been validated according to Commission Decision 657/2002/EC in all food and feed matrices included in the EU residue control plans; simple sample preparation protocols are also furnished without any need of SPE purification or solvent extraction. As a result, B ZERO SULFA and B ZERO TETRA HS are interesting options to carry out fast, cost-effective and easy screening analysis allowing the detection of twenty-two compounds widely used in food-producing animals.

Keywords: *sulphonamides, tetracyclines, cost-effective screening, multiresidue monitoring, master calibration curve*

R32
DEVELOPMENT AND OPTIMIZATION OF A MULTI-CLASS, MULTI-RESIDUE METHOD FOR VETERINARY DRUG ANALYSIS IN INFANT FORMULA INGREDIENTS AND PRODUCTS

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A modern multi-class, multi-residue method for detection, identification and quantitation of veterinary drugs using liquid chromatography-tandem mass spectrometry (LC–MS/MS) is a highly effective approach for residue monitoring with the ultimate goal of protecting consumers from potentially unsafe exposure to drug residues in foods of animal origin (milk, meat, poultry, seafood, egg and honey). In this study, we developed and optimized a method for over 150 compounds belonging to a variety of veterinary drug classes, including amphenicols, anthelmintics, antibiotics (beta-lactams – penicillins and cephalosporins, lincosamides, macrolides, quinolones, sulfonamides, tetracyclines, and others), antimicrobial growth promoters, antiprotozoals, beta-agonists, coccidiostats, dyes, NSAIDs, and tranquilizers. Infant formula and whey protein (an important ingredient for dairy products) were chosen to establish a robust, efficient and reliable method for screening, identification and quantitation of the included veterinary drug residues. The method development was divided into five main phases: (i) optimization of MS/MS conditions for individual compounds; (ii) optimization of LC conditions; (iii) development of the final LC–MS/MS method; (iv) sample preparation procedure development and optimization; and (v) method validation, data acceptance criteria set-up, and method implementation in routine testing. Automation of data processing and handling has been utilized across all five phases. The particular attention was devoted to mobile phase composition optimization and to comparison of different sample preparation approaches. The different concentrations of formic acid in the aqueous mobile phase or different ratios of acetonitrile and methanol in the organic mobile phase were evaluated to achieve a well-distributed elution profile and minimum analyte interferences. The sample preparation optimization was divided into three stages: (i) comparison of extraction procedures; (ii) comparison of different clean-up options (such as hexane defatting, dispersive SPE clean-up, or supported liquid extraction); and (iii) establishment of the sample extract dilution scheme.

Keywords: *veterinary drug residues, multi-class, multi-residue method, Infant formula, LC–MS/MS*

R33
DETERMINATION OF VETERINARY DRUG RESIDUES IN MILK USING SPE AND LC–MS/MS WITH A POLYAROMATIC UHPLC COLUMN

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Veterinary drugs are frequently administered to food-producing animals, including dairy cows. To ensure adequate food safety and prevent any unnecessary consumer exposure to these compounds, it is vital to test milk for drug residues. Development of a multi-class, multi-residue (MMR) method can be challenging not only due to the inclusion of a large number of drugs with diverse physicochemical properties, but also on account of the complex sample matrix and the instability of certain drug classes (e.g. β-lactams, tetracyclines and macrolides). The use of a generic sample preparation procedure, such as solid-phase extraction (SPE) using a polymeric sorbent, is a suitable approach for a MMR method. This poster will outline a MMR method for the determination of 49 representative veterinary drugs in milk using a simple SPE extraction procedure followed by UHPLC–MS/MS analysis. To achieve the fast and simultaneous extraction of all the drug residues, an initial generic liquid-liquid extraction procedure using an EDTA/acetic acid buffer is performed prior to extraction on a polymeric SPE cartridge. UHPLC separation is carried out with a polyaromatic stationary phase (Selectra® DA column), which exhibits alternative selectivity to a C18 phase and is capable of improved retention of polar drugs. The method was evaluated for each compound at three concentrations (1, 10 and 100 µg/kg). For most drugs, recoveries were between 70% and 120% and reproducibility was <20%. In addition, the majority of compounds could be accurately detected at a concentration of 1 µg/kg, demonstrating that the presented method is sufficient to monitor a wide range of veterinary drugs in milk. The drugs investigated belonged to several different classes, including β-agonists, macrolides, amphenicols, sulfonamides, tetracyclines and quinolones.

Keywords: *veterinary drugs, solid-phase extraction, UHPLC–MS/MS, milk*

R34
ANALYSIS OF TRANQUILIZERS IN PORK TISSUE USING LC–MS/MS

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Tranquilizers are often used in animal production, especially in pigs, because during transport from the farm to the slaughterhouse and prior to slaughter they are under considerable stress. Poor-quality meat, called PSE (Pale Soft Exudative) and high mortality rates can be the consequence, resulting in financial losses for the farmers. Furthermore aggressive vigorous animals, such as pigs and cattle can become a danger for people who deal with them at the transport. Therefore veterinary drugs with sedative and muscle relaxant effects were administered to these animals. Even if the degradation of the substances is carried out within hours, there may be residues in meat due to an administration shortly before slaughter. In the EU the use of veterinary drugs is regulated by Commission Regulation (EU) 37/2010 establishing Maximum Residue Limits (MRL) and listing prohibited substances. While residues of Azaperone and his metabolite Azaperol, which was mainly used in pig farming, are regulated in pig muscle with 100 µg/kg as sum, the MRL of Carazolol in pig and cattle muscle is set to 5 µg/kg and no MRL is required for Ketamine and Xylazine. Due to the potential risk to consumers health Chlorpromazine is listed in the Commission Regulation (EU) 37/2010 in table 2 of prohibited substances and the Community Reference Laboratories (CRLs) recommend a ccbeta of 10 µg/kg for the analysis of Chlorpromazine and 50 µg/kg of Acepromazine, Propionylpromazine and Haloperidol in kidney. Because these substances can be harmful to consumers and tranquilizers and their metabolites have been found in slaughtered animals a quick and reliable method for the analysis of tranquilizers in meat is necessary. Until now several methods for the extraction and detection of residues of tranquilizers in pork tissue are published, based on liquid extraction followed by clean-up with solid phase extraction (SPE) and measured by LC–MS/MS. In this poster we present a quick and easy method for the analysis of residues of tranquilizers in pork tissue based on liquid/liquid extraction and detection by LC–MS/MS, which is capable of confirming and quantifying the tranquilizers, according to the EU requirements of Maximum Residue Limits (MRL), laid down in the Commission Regulation (EU) No 37/2010, and which fulfills the recommended requirements of the CRL Guidance Paper (2007).

Keywords: *tranquilizer, pork tissue, liquid/liquid extraction, LC–MS/MS*

R35
ENHANCED MATRIX REMOVAL FOR MULTI-CLASS VETERINARY DRUG ANALYSIS IN FATTY SAMPLES

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Complex samples, especially those high in fat, introduce unwanted matrix co-extractives into the analytical system which can result in chromatographic interferences, matrix effects in mass spectrometry, and accumulation in chromatographic flow paths. Concise and efficient sample preparation protocols, such as QuEChERS or protein precipitation, struggle with these complex sample extracts and either include additional cleanup steps to overcome the large amount of matrix, indiscriminately retain lipids and analytes of interest, or leave a large amount of the matrix in the sample. Agilent Bond Elut Enhanced Matrix Removal – Lipid (EMR-Lipid) is a novel matrix removal sorbent for highly selective matrix removal in complex, fatty samples without unwanted analyte retention. This work implements EMR-Lipid as a dispersive solid phase extraction (dSPE) cleanup for complex samples in multi-class residue analysis. Data will demonstrate the impact of superior clean liness on analyte recovery, reproducibility, and instrumental performance. Furthermore, comparisons between EMR-Lipid and conventionally used sorbent materials demonstrate dramatic improvements in matrix removal and analyte recovery and reproducibility. The ease of use, time and cost savings, minimal method development, and dramatically cleaner samples make this an attractive cleanup option for laboratories conducting veterinary drug analysis in complex sample types such as beef liver.

Keywords: *lipid, sample preparation, EMR, veterinary drugs, matrix removal*

R36
ANALYTICAL STRATEGY TO INVESTIGATE VETERINARY MEDICINAL PRODUCT RESIDUES BELOW THEIR EU-REGULATORY LIMITS IN MEAT

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The consumers demand for meat has grown substantially over the last decades together with a request for healthy and safer food. To ensure the protection of consumers according to the EU food law, Member States are required to control the presence of various chemical substances in feed and food by implementing national residue monitoring plans. In France, official methods to control veterinary drug residues in food are developed at the Anses reference laboratory of Fougères. These methods enable confirming and quantifying target residues in regard to their respective Maximum Residue Limits (MRL) in foodstuffs. The aim of the present study was to implement specific methods to assess the contamination profile of veterinary drugs below the MRLs in meat from different species, (i.e. chicken, pig, and cattle), and through different systems of production. Among the list of veterinary medicinal products (VMPs), antibiotics and coccidiostats are commonly used in the food-producing animal veterinary practice and have therefore been considered in the present work. The selection of antibiotics and coccidiostats of interest was based on the European Food regulations and the list of residues to monitor was selected from results of the national residue control plans. So then, the analytical strategy implemented to monitor these VMPs was supported by two official regulatory methods. A multi-antibiotic LC–MS/MS screening method was used to investigate the presence of 71 compounds or some of their targeted metabolites in meat samples of breeding animals. The analysis of coccidiostats in chicken was performed with an official LC–MS/MS method applied for screening and confirmatory purposes. Coccidiostats were investigated in chicken samples only because of their specific use as feed additives in poultry to prevent or treat the related parasitic disease. An estimation of the performance for the multi-antibiotic method below the MRL levels was performed and the performance of the methods was assessed in line with the criteria of the Decision 2002/657/CE during each of the sequences of routine analysis.

Keywords: *chemical contamination, meat, veterinary drug residues, LC–MS/MS*

Acknowledgement: *This study was funded by the French National Research Agency (ANR), project SOMEAT, Contract No. ANR-12-ALID-0004. Safety of Organic Meat. Available at http://www.so-meat.fr*

R37
QUANTITATIVE ANALYSIS AND RETROSPECTIVE SCREENING OF VETERINARY DRUGS, MYCOTOXINS, PLANT TOXINS AND OTHER UNDESIRABLE SUBSTANCES IN FEED USING LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY

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A comprehensive strategy combining a quantitative method for 77 banned veterinary drugs, mycotoxins, and plant toxins, and a post-targeted screening for 425 substances including pesticides and environmental contaminants in feed. This strategy allows developing methods that cover a wide scope of compounds with different phisico-chemical properties (multiresidue-multiclass) with high selectivity and elevated or enough sensitivity. The way to achieve targeted analysis without loss of information for the untargeted analysis is to perform a generic extraction method. The extraction procedure was developed using a QuEChERS-based extraction with an ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC–HRMS) detection. For comprehensive methods with a large scope of substances, the optimization of the spectrometric parameters, mainly those linked with the ionization step, contribute to achieve better sensitivity Optimization of the mass spectrometric settings can be achieved using a conventional approach of changing–one-factor-at-a-time (COST), or using the automatic tuning procedures of each instrument. However, a better understanding of the influence of each parameter in the response, and of the interaction between factors could be achieved using statistical design of experiments (DoE). The quantitative method was validated after previous statistical optimisation of the main parameters governing ionisation, and presented recoveries ranging, in general, from 80 to 120%, with a precision in terms of Relative Standard Deviation (RSD) lower than 20 %. The full-scan accurate mass data were acquired with and without fragmentation, both with a resolving power of 50,000 FWHM and a mass accuracy lower than 5 ppm. The limit of quantification of the method was lower than 12.5 µg kg^{-1} for the majority of the veterinary drugs and plant toxins and 20 µg kg^{-1} for ergot alkaloids. For post-targeted screening a customized theoretical database including the exact mass, the polarity of acquisition and the expected adducts was built and used for post-run retrospective screening. The analytical strategy was applied to 32 feed samples collected from farms of the Valencia Region (Spain). Florfenicol, zearalenone and atropine were identified and quantified at concentrations around 10 µg Kg^{-1}. In the post-target screening of the real samples, sulfadiazine, thrimetropin and pirimiphosmethyl were tentatively identified.

Keywords: *retrospective screening, veterinary drugs, undesirable substances, animal feed, LC–HRMS*

R38
THE ROLE OF MASS SPECTROMETRY IN FOOD SAFETY CONTROL: THE EXPERIENCE OF AN OFFICIAL CONTROL LABORATORY

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Non-steroidal anti-inflammatory drugs (NSAIDs) are used as anti-inflammatory, analgesic and antipyretic drugs in medicine and veterinary. Their action mechanism is based on the blocking of the biosynthesis of prostaglandins. NSAIDs are highly effective and extensively used, but they have some adverse side effects, such as hepatotoxicity, renal disorders or allergic reactions. To assure food safety and protect consumers, the Community Reference Laboratories (CRLs) published a list of recommended concentrations for some NSAIDs that control laboratories should monitor. The matrices to control are muscle, milk, kidney, liver and plasma. Therefore, high throughput and reliable analytical methodology is required for the effective control of NSAIDs in food from animal origin. Liquid chromatography (LC) coupled to mass spectrometry (MS) is currently the technique of choice for the analysis of NSAIDs residues. A confirmation strategy using HRMS (q-Orbitrap mass analyzer) has been established as well. We present a new method for the determination of representative NSAIDs in milk based on QuEChERS methodology followed by LC–MS/MS using a biphenyl column. The method has been validated following 657/2002/CE requirements. The variety of analytes used as NSAIDs, has been an extra difficulty for the method set up and optimization. To the best of our knowledge, this is the first time that this strategy has been applied to the analysis of NSAIDs in milk. The method is straightforward, reliable, and well suited for high throughput confirmatory analysis. The limits of quantification of the method (2.5 µg/kg) agree with the recommended concentrations. The method is currently included in the scope of the accreditation of the Laboratori de l'Agència de Salut Pública de Barcelona following ISO/IEC 17025 requirements.

Keywords: *NSAIDs, triple quadrupole, QuEChERS, milk*

R39
QUANTITATIVE DETERMINATION OF SULPHYRINE IN PIG MEAT AND KIDNEY TISSUES BY LC–MS/MS METHOD

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Sulphyrine is a drug with analgesic, anti-inflammatory, and antipyretic properties. Due to the fatal side effect in human, its use is justified only in serious situations where no alternative is available or suitable. Therefore, we should be careful when using it in food-producing animal. In addition, application of effective samples preparation process and analytical method are necessary to achieve the optimal sensitivity, selectivity and specificity for residual analysis. The purpose of this study aimed to establish the analysis method of residual sulphyrine (metabolite: 4-methylamino antipyrine (MAP)) in pig meat and kidney tissues by LC–MS/MS. Meat and kidney tissues were homogenized (5 g) and spiked with sulphyrine at the concentration 20 ng/g (n=3). The samples preparation process was modified with preparation process for sulphyrine in meat of Korean Food Standards Codex (KFSC). Concentration of 20 ng/g was were chosen to evaluate the recoveries in both of tissues. Analysis was performed by Shimadzu Nexera LC interfaced to an ABsciex QTrap 6500 mass spectrometer (LC–MS/MS). The chromatographic column was a Atlantis dC18 (2.1 mm × 100 mm, 3 µm), the column temperature was 40℃ and the flow rate was 0.4 ml/min. The injection volume was 10 µl. The mobile phase consisted of 10 mM ammonium acetate in water (A) and acetonitrile (B), and the gradient was used as mentioned below: 5% B (0~2 min) - 95% B (5~7min) - 5% B (7.01–10min) The source conditions were optimized to obtain two identification points (precursor: 218.1, product: 56.1, 96.9 *m/z*). Concentration response showed linearity within the concentration ranges (muscle: $R^2 > 0.995$, kidney: $R^2 > 0.999$). The limit of quantification (LOQ) and detection (LOD) was resulted from external standard curve for muscle samples. The LOD and LOQ were 4.15 and 12.57 ng/g. Recoveries of sulphyrine in kidney were lower (51.3– 64.6%) than meat (88.7–91.5). Because of low analytical recoveries, the LOQ and LOD was resulted from the regression of the surrogate calibration curve for kidney samples, The LOD and LOQ were 7.72 and 23.40 ng/g, respectively. (The maximum residue limit of sulphyrine (major metabolite: MAP) is 0.1 ppm (100 ng/g) in KFSC). The coefficient of variation (CV, n=3) observed were 11% in muscle and 12% in kidney. The surrogate standard curve was applied to reduce effects of the lower recoveries of sulphyrine analysis for pig kidney samples. According to the obtained results, quantitative determination of aminopyrine with surrogate standard was successfully applied in pig kidney when the analytical recoveries were not sufficiently high. This method may be applied for the screening analyses in residues monitoring for sulphyrine in kidney tissue.

Keywords: *sulphyrine, pig, LC–MS/MS*

R40
DETERMINATION OF GESTAGENS IN KIDNEY FAT BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Acetylgestagens, such as medroxyprogesterone acetate, melengestrol acetate, megestrol acetate and chlormadinone acetate, are synthetic progestagens that can be used as growth promoters for livestock. In the European Union their use in food producing animals has been prohibited by the by Council Directive 96/22/EC. For effective control of illegal use of hormones, very sensitive analytical methods are required, since residues can be present at very low concentrations. The matrix of choice for monitoring of these compounds is kidney fat, because gestagens are lipophilic and accumulate in adipose tissues. The aim of this study was to develop a rapid and sensitive LC–MS/MS confirmatory method for the detection of four gestagens in kidney fat. Gestagens were extracted from fat sample with petroleum ether, extract was defatted by freezing and centrifugation, and then purified on a C18 SPE columns. The analyses were carried out by LC–MS/MS on a QTRAP5500 instrument in positive Electrospray Ionisation mode. Chromatographic separation of gestagens was performed on an Inertsil® ODS-3 (150 × 2.1mm, 3 µm) column using isocratic elution of mobile phase consisting of acetonitrile/ methanol/ water. The parameters such as recovery, repeatability, reproducibility, linearity, specificity, decision limits, and detection capabilities were investigated during the validation. The method was characterised by a good recovery (from 84 to 117%) and correct precision (CV < 25%) due to the utilization of internal standards for each compound. The values of the decision limit CCα and the detection capability CCβ for individual gestagens are found to be below the recommended concentrations set at 1–5 µg kg^{-1} and were in the range 0.15–0.30 µg kg^{-1} and 0.25–0.50 µg kg^{-1}, respectively. The specificity studies showed no interferences in the range of the retention times of analytes. The method fulfils the criteria of Commission Decision 2002/657/EC for confirmatory method in whole range of applicability. Identification of gestagens was based on retention time relative to the internal standard and the ion ratios of product ions.

Keywords: *acetylgestagens, liquid chromatography, mass spectrometry*

R41
DETERMINATION OF A SINGLE METHODOLOGY FOR THE ANALYSIS AND QUANTITATION OF MULTI-CLASS VETERINARY DRUGS IN DIFFERENT ANIMAL MATRICES USED FOR CONSUMPTION

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The quantification of over 200 multi-class veterinary drugs from different meat products usually involves a series of different extraction methods with either SPE or LLE. Each requires substantial time in both sample preparation and analytical run time with multiple HPLC and mass spectrometer methods. A new robust method, utilizing a single chromatography run and a triple quadrupole mass spectrometer is described in this poster. It can be used to quantitate over 200 vetdrugs by LC/MS in a variety of matrices. In addition, the instrumental method can be set up to perform targeted screening of samples. 2 µL injections of extracted meat (chicken, cow, pig and fish) containing many veterinary drugs were injected onto a C18 reverse phase column. Compounds of interest were separated and eluted using a standardized gradient elution profile. Prior to elution of the analytes, the MS valve was actuated to switch to waste, therefore keeping the MS source free of m atrix contamination. A high performance triple quadrupole mass spectrometer with a heated electrospray source (HESI), was used to analyze the compounds of interest in positive and negative ionization, and the data are collected, analyzed, and reported using customized software. To test the assay, standard curves with seven points were prepared in different matrices covering the range of 10 pg/mL (ppt) to 1 µg/mL (ppm). A minimum of two ions were monitored, one for quantitation and the other for qualification. The calibration curves were linear over the ranges described above. The columns showed no deterioration in quality or performance when analyzing the different matrices one after the other. To maximize instrument robustness, a divert valve and sweep gas was utilized to prevent contamination of the ion source.

Keywords: *quantitation, multi-veterinary drugs, LCMS, triple quadrupole, targeted screening*

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LAST MINUTE POSTERS

LM1
CONFIRMATION AND TYPING OF SALMONELLA BY GENOME SEQUENCE SCANNING IN PRESUMPTIVE POSITIVE FOOD SAMPLES

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INTRODUCTION: Safe production, distribution and storage of foods are a difficult responsibility of the food industry that requires rapid and accurate detection of foodborne pathogens. Pathogenetix has developed an automated system for molecular serotyping of bacterial pathogens based using Genome Sequence Scanning (GSS) technology. Resolution system extracts gDNA, performs restriction digest and labels long (>90 kb) DNA fragments with sequence-specific fluorescent probes. These DNA molecules are then stretched in a microfluidic device and interrogated by laser light obtaining optical maps of fluorescent probes binding. Optical maps are defined by the underlying genomic DNA sequence and are compared to a database for strain identification.
METHODS: We spiked 25 g of ground beef or fresh spinach with < 5cfu of one of the following Salmonella strains: 3 each of Enteritidis and Typhimurium and 1 each of Newport, Javiana, Montevideo, and Heidelberg. In one set of experiments, we also included 10-fold excess of microbes from cow feces as competing background flora. All experiments were repeated six times. Samples were enriched in BPW with Salmonella supplement (Biomerieux) for 18 h at 42°C and analyzed by GSS. Total aerobic counts and Salmonella were measured on TSA and XLD agars, respectively, for enrichment control. RESULTS: In this study we demonstrate typing of Salmonella in food matrices following enrichment for a standard screening assay. Of the 240 samples spiked with Salmonella, 235 were confirmed to be positive for the presence of the correct serovar by GSS analysis. Salmonella was not detected in five samples (4 in spinach, 1 in ground beef) due to poor enrichment confirmed by plate count. No false positives were detected from any of the 24 unspiked samples.
CONCLUSION: GSS performed sub-typing Salmonella serovars in two different matrices directly from enriched cultures even in the presence of other bacteria.

***Keywords**: molecular serotyping/straintyping, Salmonella, rapid confirmatory test, food safety*

LM2
STRAIN TYPING OF BIG 7 STECS AND DIFFERENTIATION FROM STX AND/OR EAE BIG 7 SEROGROUP E.COLI BY GENOME SEQUENCE SCANNING

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INTRODUCTION: Genome Sequence Scanning (GSS), a single molecule detection technology developed by PathoGenetix, is a bacterial strain typing tool. USDA FSIS has identified *E. coli* O157:H7 and stx+ and eae+ strains of serogroups O26, O45, O103, O111, O121 & O145 (BIG 7) as adulterants in certain meat products. However, high false-positive rates of PCR-based screening tests pose a major challenge to the reliable detection of BIG 7 STECs in foods. This study evaluated the ability of GSS to differentiate the BIG 7 STEC strains from other *E. coli* strains including those sharing the BIG 7 O-antigen but lacking stx and/or eae virulence genes.
METHODS: Genomic DNA from representative E. coli strains was extracted, digested with a restriction enzyme and labeled with sequence-specific fluorescent tags using an automated sample preparation module. The DNA fragments were linearized and their fluorescence measured in a microfluidic detection device. Fluorescent sequence-specific maps, or "barcodes" were generated. Barcodes of all strains were then compared using proprietary algorithms that compute a similarity parameter, which was used to cluster strains on a GSS- generated tree. Barcodes from whole-genome sequenced strains were calculated in-silico and added to the tree.
RESULTS: A collection of more than 500 strains and genome sequences, representing different pathogenic, commensal and laboratory strains of E. coli was used to test the strain typing capability of GSS. In general, the BIG 7 serogroup STEC strains formed serotype-specific clusters distinctly separated from those formed by other E. coli strains. Strains that share the BIG 7 O-antigen but lack stx and/or eae genes clustered away from the BIG 7 STEC groups, often in pathotype-specific clusters, indicating independent parallel evolution of serogroups/serotypes into different pathotypes. A minority of the strains lacking stx and/or eae genes, presumably spontaneous deletion mutants, clustered with the respective BIG7 STEC strains.
CONCLUSION: The results show that GSS can accurately identify the stx+ eae+ lineages of BIG 7 STECs and reliably differentiate them from other *E. coli* strains. GSS used as a rapid confirmatory test on potential-positive samples can facilitate effective implementation of food safety regulations.

***Keywords:** BIG 7 STECS, stx+ eae+ lineages, molecular serotyping/straintyping, rapid confirmatory test, Salmonella, E-Coli, Listeria, Pathogens*

LM3
A NOVEL, SIMPLE, EFFICIENT AND DIRECT METHOD BASED ON ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE SIMULTANEOUS QUANTITATION OF VARIOUS FATTY ACIDS IN OLIVE OILS OF DIFFERENT ORIGINS

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A simple, fast, highly efficient and direct method using ultra-performance liquid chromatography coupled to mass spectrometry has been established for the simultaneous separation, identification and quantitation of a few saturated and unsaturated fatty acids in olive oils from various countries. No sample pretreatment techniques were employed such as extraction or derivatization for the analysis of target acids from oil samples, as the oil samples were just diluted, filtered and then directly injected to the instrument. The chromatographic separations of all target fatty acids were achieved on a Hypersil Gold C18 column of particle size 1.9 µm, 50 × 2.1 mm I.D, while the gradient elution using a binary mobile phase mixture of acetonitrile and water at a flow rate of 1.5 mL/min was adopted for achieving optimum separations. The identification and quantitation of target compounds was accomplished using selected ion reaction monitoring mode. The recoveries of the fatty acids were obtained higher than 89% with good validation parameters; linearity (r^2>0.992), detection limit between 0.09 and 0.24 µg/ml, run to run and day to day precisions with percent relative standard deviation lower than 2.4% at both low (1 µg/ml) and medium (10 µg/ml) concentration levels. The total content of fatty acids in each individual oils was found in the range of 472.63 to 7751.20 µg/ml of olive oil, while oleic acid was found to be the major fatty acid among all analyzed oils with the amount 3785.94 µg/ml (maximum) in Syrian olive oil. The obtained validation parameters confirm that the proposed analytical method is rapid, sensitive, reproducible and simple and it could be applied for the successful evaluation of fatty acids in various oils and other matrices. All the fatty acids were efficiently eluted in a time of less than 8 min with well resolved peaks by employing the proposed method.

Keywords: *ultra performance liquid chromatography, mass spectrometry, fatty acids, olive oils*

LM4
BEHAVIOR OF PESTICIDES IN FIELD-SPRAYED LEAFY VEGETABLES DURING PARBOILING

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The reduction rate of pesticide residues on spinach (bifenthrin, metalaxyl, procymidone), chard (bifenthrin, imidacloprid) and mallow (bifenthrin, chlorpyrifos, imidacloprid) were tested on each step of washing and boiling (spinach: 1, 3, 5 min., chard: 3, 6, 9 min., mallow: 10, 20, 30 min.). The reduction rates of bifenthrin and procymidone by washing were 58~64% and 82%, and these were not changed significantly after boiling. In case of imidacloprid, the rates showed 43% on chard and 12% on mallow by washing, and these were highly increased to 94 % after boiling. And the reduction rate of metalaxyl and chloropyrifos were 69% and 11% by washing, and 96~98% and 77~79% by boiling. Specifically we monitored the pesticide residues on both boiled vegetable and its water because there are used to cook as soup in Korea. The total residual amounts of imidacloprid and chloropyrifos were effectively removed on both boiled mallow and its water (12 % → 34~40%, 11% → 76~79%), however, the other tested pesticides were not changed on pesticide residues when calculated with total amounts on boiled vegetable and its water. These explained the other pesticides were just moved vegetable to water by boiling.

Keywords: *pesticide, residue, parboiling, leafy vegetable*

LM5
COMPARATIVE THE EFFICIENCY OF SPE CLEAN-UP PROCESS APPLIED QUECHERS METHOD

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Recently, multi-residue pesticide batch analysis have been using universally for multi-residue analysis adapting in Korean food code and QuEChERS method. However, STQ (Solid phase Technique with QuEChERS) method was developed to improving clean-up effect and shorten the working hours. This study was conducted to compare with the multi-residue method in Korean food code and QuEChERS method for validated accuracy, reproducibility and efficiency. Total 45 selected and targeted pesticide were analyzed by GC and 5 of them were crops (apple, potato, green pepper, rice, soy bean). R^2 values were calculated in the standard calibration curves of three analysis methods where each method sample was over 0.990. Recovery tests were performed by three times repeated in two levels and the relative standard deviation of the repeated experiments was less than 30%. Average of recoveries in the multi-residue method in Korean food code was 89.13%, QuEChERS method was 92.45% and STQ method was 85.27%. In addition, matrix effects in multi-residue method in Korean food code was 24.61%, QuEChERS method was 23.98% and STQ method showed 11.24%. STQ method is easy and highly clean-up effect in extracted the sample solution by QuEChERS method and clean-up with C18, PLS, PSA cartridge columns. Especially when, a problem occurs because the high detection limit analysis, obtain a small amount of extraction solvent with the omission of the process of concentration from the sample preparing, and to lower the limit of quantitation, therefore large volume of the sample is injected in order to compensate for this. Results of applying the STQ method using a large volume injector, a standard calibration curve showed a higher linearity than the R^2 value of 0.990, and method detection limit (Method detection limit, MDL) was 0.01 mg/kg. It showed an average recovery of 91.86% and the relative standard deviations of the three times repeated in two levels process less than 30 % and an average matrix effect was 17.90%.

Keywords: *multi-residue pesticide, QuEChERS, STQ, LVI, PTV, Matrix effect*

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LM6
ANALYSIS OF PARALYTIC SHELLFISH TOXINS, POTENTIAL CHEMICAL THREAT AGENTS, IN FOOD USING HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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A novel method for determining Paralytic Shellfish Toxin (PST) profiles in food was developed using a combination of silica and strong cation exchange (SCX) solid phase extraction (SPE) coupled to hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS). Besides the risk for natural contamination of seafood and drinking water, PSTs also pose potent threats through intentional contamination of food, due to their high toxicity and the wide distributions of toxin-producing algae. The new preparation method aim to maintain the samples' original toxin profiles by avoiding conditions known to induce inter-conversion or degradation of the PSTs. The method was evaluated for PST extraction from water, milk, orange juice, apple purée, baby food, and blue mussels (Mytilus edulis). The extracts were found to produce reproducible retention times in HILIC-MS/MS-analysis. Overall recoveries of the PSTs from tested foods by the novel method ranged from 36 to 111%.

Keywords*: Paralytic Shellfish Toxins, HILIC-MS/MS, Silica-SPE*

LM7
MULTIRESIDUE ANALYSIS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN MILK BY QUECHERS AND LC-MS/MS

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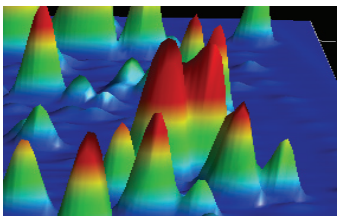
Mass spectrometry (MS) is an essential tool in food safety control laboratories. The confirmatory analysis of residues of pesticides, veterinary drugs, biotoxines, etc.is mostly based on chromatographic techniques coupled to MS. Decision 2002/657/EC as well as SANCO 12571/2013 guidance documents, all concerning methods for residue analysis in food, were pivotal for the widespread implementation of triple quadrupole spectrometers (QqQ) in official control laboratories. Thus, QqQ instruments are extensively used and have currently become workhorses in routine analysis. While sensitivity is essential to analyze low concentration levels, selectivity is also of paramount importance to assure reliable results and to avoid false positive/negative results. Although QqQ provides excellent selectivity, food samples are highly complex, and some difficulties may arise concerning confirmation criteria as established in Decision 2002/657/EC or SANCO/12571/2013. High resolution (HR) MS offers an outstanding performance, and is an attractive approach for the confirmatory analysis of residues and contaminants in food samples. HRMS hybrid instruments, such as quadrupole-Orbitrap, start to be introduced as analytical systems in food safety control laboratories. This instrumentation is a very valuable tool to confirm doubtful results obtained with low resolution MS/MS. In conclusion, HRMS is emerging as a good approach for combining the qualitative and quantitative analysis together, minimizing the effect of the matrix and also the potential inaccuracies in quantitation by liquid chromatography tandem mass spectrometry. In this communication we present some analytical strategies based on low and high resolution MS/MS used in an official control laboratory, and some examples are shown.

Keywords: *HRMS confirmation, Triple quadrupole, Decision 2002/657/EC*

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